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OF
PROGRAM ACTIVITIES
NATIONAL CANCER INSTITUTE
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TABLE OF CONTENTS

DIVISION OF CANCER TREATMENT

DIRECTOR

		Page
Introduction		1
Code for Division of Cancer Treatment Program Logic		5
Table I	Analysis of Contract Activities	7
Table II	Analysis of Contracts by Activity	9
Table III	Analysis of Activities by Contract	37
Table IV	Description Contracts	75

ASSOCIATE DIRECTOR FOR PROGRAM

Project Reports:

NCI-3409	Leukocyte Kinetics and Characteristics of Cell Proliferation	129
NCI-6401	(a) Data Processing Systems for Scientific and Management Information in Division of Cancer Treatment	134
	(b) Simulation Study of Preclinical Development of Anticancer Drugs	
NCI-6402	Tumor Growth Kinetics and Chemotherapy	137
NCI-7025	Biomedical Engineering for Intramural Research, DCT	142
NCI-7070	Automated Grain Counting/Densitometer	150
NCI-7071	Automatic Cell Analysis	156
NCI-7085	Kinetic (Quantitative) Studies on Tumor Cell and Biomarker Interactions; and on Cell Age, Cell Size, and DNA Content Distributions and Their Implications to Cancer Chemotherapy	160

ASSOCIATE DIRECTOR FOR DRUG RESEARCH AND DEVELOPMENT Page

Summary Report 165

Project Report:

NCI-3575 Chemotherapy Research Including Fundamental Biological and Biochemical Investigations of Antitumor Agents 173

Mammalian Genetics and Animal Production Section 191

Publications Section 205

Drug Development Branch

Summary Report 209

Chemical and Drug Procurement Section 243

Natural Products Section 257

Drug Design and Chemistry Section 265

Project Reports:

NCI-3580 Chemical Research in Development of New Anticancer Drugs 266

NCI-3581 Analytical Chemical Chemistry of New Anticancer Drugs 274

Clinical Drug Distribution Section 277

Project Report:

NCI-3584 Research in Dosage Form Development of New Antitumor Drugs 283

Chemical and Drug Records Section 287

Data Processing and Computer Systems Development Section 291

Scientific Records Section 295

Publications by Contractors 299

Publications by Staff 305

Drug Evaluation Branch Page

Summary Report 307

Publications by Staff 325

Publications by Contractors 327

Automated Information Section 329

Biochemistry Section 335

Molecular Biology and Methods Development Section 345

Project Reports:

NCI-3579 Effects of Chemotherapeutic Agents on L1210 Cell Cycle Progression and L1210 Cell Nuclear Proteins 351

NCI-3583 Studies on Modification of Division Patterns and Immunogenecity of Neoplastic Cells by Interaction of their Surfaces with Microenvironmental Macromolecules 357

Screening Section 361

Project Report:

NCI-3578 Basic Studies for Drug Evaluation Utilizing Antitumor Screening 376

Laboratory of Experimental Chemotherapy

Summary Report 379

Project Reports:

NCI-7100 Tumor Biology and Chemotherapy 382

NCI-3577 Immunochemotherapeutic Studies with Various Murine Lymphomas 393

ASSOCIATE DIRECTOR FOR EXPERIMENTAL THERAPEUTICS

Summary Report 401

Project Reports:

NCI-3500 The Distribution of Drugs Between Blood, Brain and Cerebrospinal Fluid 407

NCI-3516 Biochemical, Pharmacologic, Toxicologic and Chemotherapeutic Aspects of Selected Antitumor Agents 413

Laboratory of Toxicology

Page

Summary Report 417

Project Reports:

NCI-3518 L-Asparagine: Synthesis, Biological Role, and Hydrolysis Streptozotocin: Alkylation of Nucleic Acids 425

NCI-6110 The Pathology of Drug-Induced Alterations in Animals and Man 433

NCI-6113 The Detection, Quantitation, Evaluation and Prediction of the Pathologic Effects of Antineoplastic Agents, Especially on the Hematic System, and Studies of Other Conditions of Host-Defense Failure 440

NCI-6122 Drug Interactions and Other Factors Influencing the Physiological Disposition and Toxicity of Drugs 443

NCI-6123 Computer Methods for Quantification of Structure Activity Relationships 450

Laboratory of Chemical Pharmacology

Summary Report 453

Project Reports:

NCI-3502 Studies on Folic Acid Antagonists 463

NCI-3505 Potential Embryotoxic, Carcinogenic and Other Adverse Effects of Anticancer Agents 470

NCI-3506 Pharmacology and Disposition of Alkylating Agents and Other Antitumor Drugs 474

NCI-3509 Carcinogenesis, Chemotherapy and Biological Markers in Primates 479

NCI-6108 Studies on Mechanism of Action, Mechanisms of Resistance and Anti-Metastatic Activity of Anticancer Agents 487

NCI-6116 Studies with Hepatic Aldehyde Oxidase, Xanthine Oxidase, and Other Drug-Metabolizing Enzymes from Mammalian Liver Cytosol 491

Page

NCI-6120 Kinetic Profile on Drug Action *In Vivo* 494

NCI-6121 Purification and Properties of Carboxypeptidase G_1 497

NCI-6124 Biochemical and Pharmacological Studies Designed to Improve Clinical Cancer Chemotherapy: 1) Biological Markers of Malignancy; 2) Enzymatic Reactions which may be Exploited for the Development of Better Chemotherapeutic Agents 499

NCI-6125 Pharmacology of 2,6-Dimethyl-4-benzyl-4-demethylrifampicin (NSC-143414) and other Inhibitors of Viral RNA-directed DNA Polymerase 503

NCI-6126 Purification and Properties of Cytidine Deaminase 506

Laboratory of Tumor Cell Biology

Summary Report 509

Project Reports:

NCI-6117 Studies on Mechanisms Leading to Neoplastic Transformation; On Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells; On New Approaches to Cancer Chemotherapy; and on the Development of Biochemical Markers of Neoplastic Cells 513

NCI-6119 Cytogenetic Studies: in human neoplastic, hematologic and congenital diseases; to study T and B lymphocytes to determine the origin of stem cell by using the diffusion chamber technique in mice; to study T and B proliferation,by using an *in vitro* chimeric system created by separation and subsequent cross-recombination of T and B lymphocytes from HL-A genotype-identical, MLC-negative siblings of opposite sex; to explore possible viral genome incorporation by using *in situ* hybridization in normal and leukemic bone marrow cells; to continue to study chromosomal aberrations and the possible correlation between the development of leukemia and total body irradiation in long surviving lymphoma patients; to determine the survival of transfused lymphocytes in patients with numerous blood transfusions; to study the survival of bone marrow transplantation in humans, monkeys, and dogs; to evaluate chromosomal abnormalities caused by MTX in psoriasis treatment; to observe *in vivo* and *in vitro* effects of drugs and viral transformation. 522

Laboratory of Molecular Pharmacology Page

Summary Report 525

Project Report:

NCI-6104 Effects of Chemotherapeutic Agents on DNA 527

ASSOCIATE DIRECTOR FOR MEDICAL ONCOLOGY

Summary Report 533

Project Report:

NCI-3423 *In Vitro* Marrow Granulocyte Culture 542

NCI-VA Medical Oncology Branch

Project Report:

NCI-3024 Chemotherapy of Cancer 549

Medicine Branch

Project Reports:

NCI-3403 Clinical Trials and Miscellaneous Clinical Investigations 587
NCI-3404 Immunologic Aspects of Cancer 608
NCI-3405 Hematologic Investigations 611
NCI-3420 Kinetics of Cellular Proliferation 616
NCI-6500 Clinical Pharmacology--Mechanism of Action, Physiologic Disposition, Metabolism and Toxicity of Anticancer Agents 620

Medical Breast Cancer Service

Project Reports:

NCI-6700 Clinical Program in Breast Carcinoma 625
NCI-6701 Factors Controlling Lymphocyte Reactivity *In Vitro* 632

Pediatric Oncology Branch

Project Reports:

NCI-3422 Study and Treatment of Acute Leukemia, Solid Tumors of Childhood, Hepatocellular Carcinoma and Kaposi's Sarcoma 635

Page

NCI-6308 Biostatistical Information Systems Unit 643

NCI-6810 Chemo-immunotherapy 647

NCI-6820 Blood Component Transfusion and Bone Marrow Transplantation 653

NCI-6830 1) Studies on Integration and Genetic Organization of Oncogenic Papovavirus, SV40, using Adenovirus 2-SV40 Hybrid Virus 667

2) Studies on Acute Infection, In Vivo Tumorigenesis, and In Vitro Transformation Induced by Various DNA and RNA Tumor Viruses: Viral and Host Components as Assayed by Biochemical and Biophysical Techniques

3) Studies on AKR Murine Leukemia Virus Genome and Activation of RNA Tumor Viruses by Halogenated Pyrimidines

4) Infectious Complications of Malignancy: Natural History, Prevention, Diagnosis, and Therapy

ASSOCIATE DIRECTOR FOR BALTIMORE CANCER RESEARCH CENTER

Office of the Associate Director

Summary Report 683

Clinical Branch

Summary Report 685

Microbiology Section:

NCI-6027 Microbiological Services for Clinical and Research Studies for Cancer Patients at BCRC and at NIH, Clinical Center by Contracted Laboratory Facilities 687

NCI-6039 A Computerized Microbiology Reporting and Patient Information System for Use by a Distantly Located Contracted Laboratory Facility 690

NCI-6219 Studies Directed Toward Elucidation of Cancer Patient Microbial Flora, Colonization by Specific Bacterial Genera and Infection 693

Page

NCI-6223 Studies on *Pseudomonas aeruginosa* in a Cancer Research Center 699

NCI-6254 Research Activities Directed Toward Improvement of Microbial Laboratory Techniques 704

Medical Oncology Section:

NCI-6217 Prevention of Infections in Patients with Cancer Including Evaluation of Protected Environments and Surveillance Cultures 709

NCI-6230 Diagnostic Laparotomy and Splenectomy for Staging Hodgkin's Disease 713

NCI-6259 Extension of the Computerized Problem-Oriented Medical Information System (PROMIS) into the Cancer Treatment Area 716

NCI-6263 Protocol for Staging and Treatment of Lymphosarcoma and Reticulum Cell Sarcoma 721

NCI-6264 Clinical and Autopsy Observations of Infectious Diseases in Cancer Patients 723

NCI-6266 Therapy of Infection in Patients with Cancer 728

NCI-6275 Cell Transfusion Therapy 731

NCI-6276 The Chemotherapy of Metastatic Testicular Carcinoma 735

NCI-6277 The Chemotherapy of Adult Acute Leukemia 737

NCI-6278 Determination of Serum Alpha Fetoprotein and Plasma Carcinoembryonic Antigen, Regan Isoenzyme, and Other Markers, in Patients with Malignant Disease 743

NCI-6279 Initial Clinical and Pharmacological Evaluation of Adriamycin 745

NCI-6280 Combination Chemotherapy for Stage III-B and IV Hodgkin's Disease 748

NCI-6293 A Teaching and Consultation Service in Medical Oncology 750

NCI-6295 Census: A Remote Access Patient Information Storage and Retrieval System 753

		Page
NCI-6297	Streptozotocin Therapy for Malignant Carcinoid and Pancreatic Islet Cell Tumors	756
NCI-6298	Streptozotocin in Therapy of Lymphoproliferative Disorders	758
NCI-6299	Therapy of Patients with Metastatic Sarcoma	760
NCI-6900	Radiation Alone vs. Radiation Therapy Followed by Combination Chemotherapy in Treatment of Hodgkin's Disease	763
NCI-6913	Diagnosis of Unexplained Pulmonary Infiltrates in Cancer Patients. A Pilot Study Utilizing Transtracheal Bronchial Brushing	765
NCI-6914	Intensive Supportive Care Alone vs. Intensive Supportive Care Plus Radiotherapy for Localized Inflammatory Lesions in Patients with Acute Leukemia	767
NCI-6915	Staging Laparotomy and Combined Modality Therapy in Stage I-A and II-A Lymphocyte Predominant, Modular Sclerosing, and Mixed Cellularity Hodgkin's Disease	769
NCI-6916	Pilot Protocol for Treatment of Patients with Stage III and IV Diffuse Histiocytic Lymphoma	771
NCI-6917	Cyclophosphamide, Adriamycin, Vincristine, and Prednisone vs. Cyclophosphamide, Vincristine, and Prednisone for Disseminated Non-Hodgkin's Lymphoma	773
NCI-6918	Streptozotocin, Bleomycin, CCNU, and Adriamycin for Treatment of Advanced, Refractory Hodgkin's Disease	775
NCI-6919	Initial Drug Therapy for Metastatic Renal Cell Carcinoma	777
NCI-6920	5-Azactidine vs. Guanazole for Therapy of Previously Treated Adult Acute Non-Lymphocytic Leukemia	779
NCI-6921	Adriamycin Therapy for Advanced Mycosis Fungoides	781
NCI-6922	Epidemiologic Investigations of Possible Social Clustering Among Patients with Leukemia and Lymphoma	783

Neurosurgery Service: Page

Project Reports:

NCI-6024 Development of Neurosurgical Service with Primary Interest in Treatment of Brain Tumors at BCRC,NCI 785

NCI-6202 Murine Intracerebral Melanoma Study 789

NCI-6204 Toxicity of Intracerebral Chemotherapy in Monkeys 791

NCI-6268 Radio-labeled Distribution of Methyl CCNU in Dogs 793

NCI-6282 The Brain Tumor Study Group 795

NCI-6283 An Evaluation of 4'Demethyl-Epipodophyllotoxin-B-D Thenylidene Glucoside (PTG) in a Phase II Study Treating Primary Intracranial Neoplasms 798

NCI-6286 Methyl CCNU: Phase I Toxicologic and Neuropharmacologic Evaluation and Phase II Therapeutic Study 800

NCI-6904 Studies on Antitumor Activity of Methyl CCNU and Related Compounds at Subcellular Level 802

NCI-6905 Intravenous Methyl CCNU in Malignant Central Nervous System Neoplasms 805

NCI-6906 Intravenous CCNU Phase I Study 807

NCI-6907 Colony Stimulating Factor (CSF) and Colony Forming Capacity (CFC) from Patients with Malignant Neoplasms, and Effects of Chemotherapy Upon CSF and CFC 809

NCI-6908 Intravenous Methyl CCNU (NSC-954410) and PTG (NSC-122819) in Treatment of Central Nervous Neoplasms 811

NCI-6909 Phase I Evaluation of Intrathecal N,N'N" Triethylethiophosphoramide (Thiotepa NSC-6396) in Meningeal Neoplasms 813

NCI-6910 *In Vitro* Metabolism of CCNU and Methyl CCNU 815

NCI-6911 *In Vivo* Metabolism of 3-Cyclohexyl Substituted in 1-Chloroethyl 1-Nitrosoureas 817

Patient Care Pharmacy Service Page

NCI-6043 Automated Monitoring of Drug Therapy 819

NCI-6236 Role of Pharmacist in Obtaining a Patient's Drug and Allergy History 821

NCI-6237 Development of Patient Care Pharmacy Service 823

NCI-6238 Development of Unit Dose Service and Preparation of Parenteral Drugs by Patient Care Pharmacy Service 825

NCI-6912 Antineoplastic Drug Displays for Computerized Problem-Oriented Medical Information System (PROMIS) 827

Radiation Therapy Service

NCI-6023 Radiation Therapy Service 829

NCI-6207 Radiological Physics. A Special Service which is Supplied for Research Purposes 831

NCI-6270 Combination Chemotherapy and Radiotherapy in Treatment of Hodgkin's Disease 833

Laboratory of Pharmacology

Summary Report 835

Biochemistry Section

Project Reports:

NCI-6012 Biochemical Pharmacology of Cancer Chemotherapeutic Agents 838

NCI-6014 Studies on Cellular Control Mechanisms Affecting Cell Growth and Differentiation 840

NCI-6016 Pharmacodynamics of Cancer Chemotherapeutic Agents 843

NCI-6241 Clinical Pharmacology of Cancer Chemotherapeutic Agents 845

Enzymology and Drug Metabolism Section

NCI-6210 Development of Specific RNases as an Aid in Sequencing of tRNA from Murine Leukemia Viruses 847

NCI-6242 Polyamines and Cancer; Functional Significance 849

Page

NCI-6245 Studies on Human Platelet Carbohydrate Metabolism: 1) The Effect of Cyclic AMP; 2) Interactions of Thrombin and Nucleotides and Their Effects on Clycolysis; 3) Effects of Storage and Various Drugs on Glycogenesis and Glycogenolysis 851

NCI-6247 Purification and Study of Bacterial and Mammalian Nucleases with Differing Residue Specificities 852

NCI-6287 Studies on Glycosidases of Human Platelets 854

NCI-6288 Polyamine Biosynthesis in Rapidly Growing Tissues 855

NCI-6289 Purification of S-adenosyl-L-methionine Decarboxylase from Rat Liver and from other Biological Sources 857

NCI-6291 Polyamine Metabolism in Spleens and Livers of Leukemic Mice. Effects of Potential Anti-cancer Agents 859

NCI-6902 The Characterization of Viral RNA Isolated from AKR Murine Leukemia Virus 861

NCI-6903 Development of Specific DNases as an Aid in Sequencing of DNA from Various Viral Tumors 863

NCI-6925 Studies on Metabolism, Function and Storage of Human Blood Platelets 865

NCI-6926 Role of Polyamines in Regulating Activity and Specificity of Various Nucleases 866

NCI-6927 Properties of Enterobacter Nuclease and Its Interaction with Polyamines 868

Immunology and Cell Biology Section

Project Reports:

NCI-6020 Mechanisms of Human Lymphocyte Reactivity to Non-Self 871

NCI-6248 To Determine Effect of Freezing on Transformation of Lymphocytes in Mixed Lymphocyte Reaction and in Response to Specific Antigens and PHA 874

NCI-6249 Search for Antigen-Antibody Complexes in Renal Glomeruli of Tumor Patients 876

Page

NCI-6274 Production of Tumor Specific Antibody in Xenogeneic Species Utilizing Blocking Antibody: Characteristics of Reactivity with Complement System 878

NCI-6292 Immune Adjuvants 879

Research Pathology Section

Project Reports:

NCI-6250 Ultrastructural and Tissue Culture Studies of Acute Leukemic Cells of Patients Before and During Treatment and Donor Granulocytes Harvested for Transfusion Therapy of These Patients 881

NCI-6272 Studies of Myelomonocytic Leukemia in an Animal Model System: Electron Microscopy and Biological Experiments 883

NCI-6923 Fine Structure and Cytochemistry of Human Neoplastic Solid Tumors 886

NCI-6924 Ultrastructural Studies of Aberrant Cells in Possible Preleukemic States 887

ASSOCIATE DIRECTOR FOR CANCER THERAPY EVALUATION

Summary Report 889

Literature Research Section 905

Combined Modality Branch 907

Investigational Drug Branch 949

Drug Liaison and Distribution Section 977

ANNUAL REPORT

DIVISION OF CANCER TREATMENT

Seymour Perry, M. D.
Acting Director

In fiscal year 1974, the Division of Cancer Treatment continued to implement program changes initiated in the previous year. The new program thrust evolved as an outgrowth of research developments in the last few years and was given impetus by the National commitment to cancer research.

A great deal has been learned about cancer and the management of these diseases and in fact, many patients with the more rapidly growing tumors can now be given a normal life expectancy either by drugs alone or in combination with surgery or x-irradiation. These tumors are the less common malignancies but the successes in this group have provided knowledge and principles which are now being applied to the more common slowly growing tumors. Some of the principles which have emerged include:

(1) different cancers have different therapeutic requirements so that treatment programs must be disease oriented rather than modality oriented

(2) unrecognized disseminated disease must be considered in designing treatment regimens, hence combinations of systemic and local treatment modalities must be emphasized in treatment and in clinical trials

(3) appropriate combinations of drugs are in general more effective than single drugs and long term intermittant administration at maximally tolerated levels is essential.

It is obvious that if a patient presents with disseminated disease (as in acute leukemia) systemic treatment is necessary. At present, the only established systemic treatment is chemotherapy although immunotherapy seems to hold important promise, perhaps in the near future. On the other hand if a patient presents with clearly localized disease, a locally effective modality such as surgery or radiotherapy should be employed. In fact, in most situations where there is no dissemination, these two modalities have a great curative potential.

However, in the common solid tumors the difficulty lies in the fact that the majority of patients present themselves with widely disseminated disease even though the detection techniques usually employed seem to indicate that the disease is still localized. Metastases may become obvious subsequently at an interval dependent upon the generation time of the malignant cells, their location, the rate of their destruction (i.e. "cell loss", the effectiveness of the immune mechanism, etc.). In some diseases, re-induction at another site may be important. In any case, it is this presence of occult disseminated disease which must be considered in designing a therapeutic regimen for a given patient.

With the foregoing considerations in mind, the DCT senior program staff evolved during the year the following general conceptual guidelines:

(1) establishment of priorities among tumors based on incidence, mortality and probability of success

(2) development of therapeutic strategies for these tumors

(3) mobilization of existing resources and development of new resources as required

(4) integration and coordination of all treatment resources.

Although surgical and radiation research are not within the current responsibilities of DCT, the strategy includes not only the continuation of the present program of drug development and drug trials but also testing independently of new surgical, radiotherapeutic, and possibly immunotherapeutic procedures. Optimal single modality regimens are to be integrated into combined modality approaches in the primary treatment of local, and regional disease and in disseminated disease when appropriate. Optimal single modality regimens will be utilized in the therapeutic approach to disseminated disease. New regimens would be developed and tested in advanced disease and those demonstrating effectiveness would be utilized in primary treatment of disseminated disease. Optimal regimens thus identified would be integrated into combined modality programs.

Although in FY 74 efforts to coordinate and pool NCI resources proceeded slowly, the groundwork for most of the DCT plan was laid during the year. An important effort in pre-clinical development and testing of drug combinations was initiated largely following up on the recommendations of a committee which had been established to deal with problems of combination chemotherapy. A group of chemotherapists and radiotherapists also met during the year to deal with research questions combining radiation and drugs both at the laboratory level and in the clinic. A scientific meeting was then held late in the fiscal year which should provide the framework for an important program of research combining these two modalities.

During the year clinical resources were expanded with support under the contract mechanism and provided for Phase I, Phase II and Phase III drug studies in breast, brain, gastrointestinal including pancreatic, testicular cancers, and ovarian cancers.

Since detection of occult disease, particularly following treatment, is one of the most critical problems in cancer therapy, important emphasis is being given to the biologic markers program. A large effort is being established at Fort Detrick which will serve as a focal point for the program and will test biologic samples from patients for markers which have already been shown to be useful.

Although the new thrust is in its early phases, there were some important observations in therapy reported during the year. In one type of lung cancer (oat cell), a response rate approximating 60% can be achieved with significant prolongation of survival utilizing a drug combination. A variety of drug combinations in advanced breast cancer have produced remission rates (partial plus complete) in the range of 50% to 60%. Many of these combinations are now being integrated into combined modality trials with surgery. The rapid advances being made in the treatment of breast cancer suggest that this tumor may be the first of the three most common tumors (breast, lung, and colon) in the United States in which patients with advanced disease will be afforded a normal life expectancy.

The Division continued to place heavy emphasis on drug acquisition and screening. The wider search for new drugs encompassing Western Europe and Japan and included under the drug exchange agreement with the USSR was continued and led to a significant increase in the number of materials screened. The introduction of the mini-screen permitted the testing of a larger number of compounds but unfortunately, analysis of the results with this new procedure suggests that there may be problems in reproducibility and that it may have to be modified or abandoned in its present form. The question is in the process of evaluation and a decision should be forthcoming in the next few months. These evolutionary problems have not affected the number of drugs proceeding toward clinical evaluation. There are currently 40 drugs passed DN2 and progressing toward clinical trial, 10 drugs in Phase I study, and thirteen IND licenses granted in 1973.

The encouraging observations in the clinic during the year were paralleled by many important findings in fundamental research aimed at elucidating cellular control mechanisms. In particular, the characteristics and properties of reverse transcriptase and the other DNA polymerases, which may have important implications for control of the neoplastic process are being defined. Such studies should lead to the design of more effective anti-tumor agents and based on a more rational basis. There are many other areas of fundamental research which need to be focussed and supported to deal with the problems of the control and cure of cancer. These include the studies of the cell membrane. the blood supply and vasculature supporting tumor growth, the chemical and hormonal regulators of cell proliferation and differentiation, and the immune mechanism at the molecular level. There are already some efforts in these areas in the DCT program but additional support is needed, provided slots and funds are available.

The enthusiasm associated with the important and exciting advances in fundamental and therapeutic research was marred near the end of the year by the sudden resignation of Dr. C. Gordon Zubrod. In 1965 Dr. Zubrod was assigned the responsibility of reorganizing the CCNSC into a comprehensive cancer chemotherapy program. His efforts led to the establishment of an integrated program in anti-tumor drug development and brought together the existing collaborative research staff on the CCNSC and certain relevant and essential laboratory and clinical units of the Institute into what was known as the Chemotherapy Program. The research strategy of this effort was built around two objectives:

(1) the search for new anti-tumor drugs and

(2) the use of drugs to achieve selective killing of tumor cells.

The structure of the Program was a radical departure at the time from the traditional organization of NCI and NIH but it provided the resources to explore new perspectives and the capability to pursue defined objectives.

In time and with passage of the National Cancer Act of 1971 the responsibilitie of the program were broadened to include all of cancer therapy. Under the leadership of Dr. Zubrod the transition was accomplished effectively and relatively smoothly. Through his efforts the National Cancer Institute became the focal point for research in the cancer treatment, not only in the United States but all over the world. His contributions were many but perhaps they can best be summarized by the statement accompanying the Albert Lasker Special Award he received in 1972: "... Dr. Zubrod has creatively guided cancer chemotherapy closer to the cure of leukemia and Hodgkin's Disease. He has helped gain for cancer chemotherapy its full acceptance as an important tool in the control of all forms of cancer".

CODE FOR DIVISION OF CANCER TREATMENT PROGRAM LOGIC

Stage	Code	Description
I	1XX	Acquisition of new agents (includes studies of cellular control mechanisms).
IIA	2AX	Determination of anti-tumor activity of new agents.
IIB	2B1	Develop acceptable experimental formulation.
IIB	2B2	Detailed evaluation of new agents - dose, route, and schedule dependency (in vivo and in vitro systems). Cell kinetics.
IIB	2B3	Initial animal pharmacology studies; evaluation for absorption and anti-tumor activity after oral administration.
IIB	2B4	Procurement of sufficient amounts of new agent for pre-clinical studies.
III	3X1	Toxicology and pharmacology in large animals (dogs and monkeys). Pharmacokinetics.
III	3X2	Production and formulation for clinical trials.
IV	4X1	Clinical Trials*- Phase I. Determination of tolerated dose. Cell Kinetics.
IV	4X2	Clinical pharmacology - initial pharmacologic studies in man associated with early clinical trials. Pharmacokinetics.
V	5XX	Clinical Trials* - Phase II. Initial therapeutic trials. Development of "markers".
VI	6X1	Clinical Trials* - Phase III. Initial eradicative trials in man. Agent used singly or in combination with other agents in comparative study. Research, development and subsequent evaluation in supportive care (protected environment, platelet and leukocyte transfusions, antibiotics, etc.)
VI	6X2	Toxicologic and pharmacologic studies in large animals and man associated with further development of a drug. Microsomal Enzyme. Molecular Biology.
VII	7XX	Clinical Trials* - adjuvant studies (radiotherapy, surgery, immunotherapy). Determination of role of agent in total care. Rehabilitation.
---	8XX	Unassigned. Includes administration, dissemination of information to medical and scientific community. Generalized D.P.

* Supportive care used as ancillary therapy should be prorated among the phases of clinical trials using such resources.

Linear Array Research:	The sequence of essential and minimum operational activities required to evaluate new agents, integrate them into a comprehensive therapy regimen and introduce them into medical practice.
Supplementary Array Research:	Research which, though not essential to operational success, contributes to methodological improvement of Linear Array activities.
Exploratory Array Research:	Research activities aimed at understanding relevant phenomena which, if successfully elucidated and exploited, could have an important impact upon cancer therapy.

TABLE I

TYPE OF ACTIVITY BY LINEAR ARRAY CLASSIFICATION		NO. OF CONTRACTS	ANNUAL LEVEL[1/]		(PERCENT)	
	TOTAL	211[2/]		39,579,524		100
STAGE I				7,813,723		19.75
1XX	Acquisition of New Agents	100	7,813,723		19.75	
STAGE IIA				6,269,323		15.84
2AX	Determination of Anti-tumor Activity	79	6,269,323		15.84	
STAGE IIB				6,842,776		17.28
2B1	Experimental Formulation Development	5	179,207		0.45	
2B2	Detailed Evaluation of New Agents	79	5,057,678		12.78	
2B3	Initial Annual Pharm. Studies	0	0		0	
2B4	Procure. of Agents for Preclinical Studies	14	1,605,891		4.05	
STAGE III				6,690,285		16.92
3X1	Toxicology and Pharm. in Large Animals	39	4,489,287		11.35	
3X2	Product. and Formulation for Clinical Trials	16	2,200,998		5.57	
STAGE IV				1,068,316		2.69
4X1	Clinical Trials-Phase I	9	611,429		1.54	
4X2	Initial Clinical Pharm. Studies in Man	8	456,887		1.15	
STAGE V				1,974,288		4.98
5XX	Clinical Trials-Phase II	40	1,974,288		4.98	
STAGE VI				5,842,402		14.77
6X1	Clinical Trials-Phase III	42	2,850,469		7.21	
6X2	Tox. & Pharm. Studies in Large Animals and Man	30	2,991,933		7.56	
STAGE VII				2,148,475		5.43
7XX	Clinical Trials-Adjuvant Studies	19	2,148,475		5.43	
PROGRAM MGT.				929,936		2.34
8XX	Administration and Information Functions	14	929,936		2.34	

[1/] Funds obligated during Fiscal 1974. Not included are funds utilized for direct purchase of clinical drugs (approximately $4,500,00), reimbursable agreements with the Veterans Administration ($1,210,000), reimbursable agreement with the Public Health Service in Baltimore ($2,500,000), and about seventeen new contracts not negotiated as of this report ($5,344,316).

[2/] Since many contracts involve more than one activity, the totals of Stages I through VII and the unassigned totals category do not correspond with the total number of contracts.

TABLE II

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE I: ACQUISITION OF NEW AGENTS	
AGRICULT., DEPT.OF (64-1)	349,000
ARIZONA BOARD OF REGENTS ARIZONA STATE UNIV. (71-2308)	48,600
ARIZONA BOARD OF REGENTS UNIV. OF ARIZONA (73-3750)	114,500
ARS/SPRAGUE-DAWLEY (71-2007)	93,756
ARS/SPRAGUE-DAWLEY (70-2044)	7,381
ASH STEVENS, INC. (71-2314)	2,231
ASSOCIATED BIOMEDIC SYSTEMS, INC. (70-2271)	128,312
BATTELLE MEM. INST (70-2008)	11,220
BATTELLE MEM.INST. (69-2251)	0
BATTELLE MEM.INST. (71-2182)	0
BELLAIRE ACRES (73-832)	3,024
BELLAIRE ACRES (73-805)	1,232
BRISTOL-MYERS CO. (69-35)	283,560
BRITISH COLUMBIA, UNIV. OF (72-3223)	67,200
CHAS. RIV. BREEDING LABS. (73-807)	1,526
CHAS. RIV. BREEDING LABS. (73-839)	3,794
CHAS.RIV.BREEDING LABS. (72-2004)	117,049
CHEM.ABSTR.SERVICE (65-1021)	350,952
COLLABORATIVE RES., INC. (66-506)	89,557

CONTRACT	DOLLAR LEVEL
STAGE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
COLLABORATIVE RES., INC. (71-2326)	51,000
DELAWARE, UNIV.OF (69-21[illegible]5)	0
DOW CHEMICAL CO. (73-[illegible]	160,000
DOW CHEMICAL CO. (71-2[illegible]	0
FLOW RES.ANIM.,INC. (73-838)	3,220
GEORGIA, UNIV. OF (71-2311)	0
HARLAN INDUSTRIES (73-844)	1,450
HARLAN INDUSTRIES (73-845)	3,561
HARLAN INDUSTRIES (72-3218)	9,450
HAWAII, UNIV. OF (73-3747)	65,000
HAZLETON LABS.,INC. (72-3704)	37,100
HORTON LABS. (73-837)	3,276
IIT RESEARCH INST. (74-3755)	23,212
IIT RESEARCH INST. (68-1021)	100,000
ILLINOIS, BOARD OF TRUSTEES OF THE UNIVERSITY OF (72-2078)	90,000
ILLINOIS, BOARD OF TRUSTEES OF THE UNIVERSITY OF (72-3208)	68,252
INDIAN CANCER RES. CENTRE (P.L.480)	0
INST.JULES BORDET (72-2053)	12,000
IST. DI RICHERCHE FARM. 'MARIO NEGRI' (73-3720)	22,424
JACKSON LAB. (74-125)	11,591

CONTRACT	DOLLAR LEVEL
STAGE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
JAPANESE FD. FOR CANCER RESEARCH (72-2054)	5,466
KANSAS, UNIV.OF (71-2010)	12,338
LAB. SUPPLY CO. (73-840)	3,719
LAB. SUPPLY CO. (73-835)	1,438
LAB.SUPPLY CO. (70-2041)	18,756
LEO GOODWIN INST. FOR CANCER RESEARCH (73-3729)	25,871
LITTLE,ARTHUR D.INC.(73-3727)	150,085
LITTLE,ARTHUR D.INC.(72-3711)	45,680
LITTLE,ARTHUR D.INC.(72-3257)	37,949
LITTON BIONETICS, INC. (72-3294)	138,160
LITTON BIONETICS, INC., (72-2062)	36,343
LITTON BIONETICS, INC., (71-2340)	700,629
MASON RES. INST. (71-2259)	12,617
MIAMI, UNIV. OF (70-2225)	3,082
MICHIGAN, REGENTS OF THE UNIV. OF (69-2004)	25,000
MICRO. ASSOC.,INC. (73-3728)	397,050
MIDWEST RES. INST. (73-3743)	324,221
MURPHY BREEDING LABS. (73-868)	1,215
N.Y. STATE DEPT.OF HEALTH (70-2035)	13,599
NEW JERSEY COLLEGE OF MED.& DENTISTRY (72-3211)	12,250

CONTRACT	DOLLAR LEVEL
STAGE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
PARKE, DAVIS & CO. (74-3754)	448,061
PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (73-3719)	521,310
PENNWALT CORP. (71-2309)	30,000
PFIZER,CHAS.& CO. INC.(73-3234)	156,210
POMONA COLLEGE (73-3703)	39,198
RESEARCH TRIANGLE INST.(69-2019)	130,150
RHODE ISLAND, UNIV. OF (71-2312)	0
SCRIPPS CLINIC & RES. FD. (72-2042)	36,849
SHEEHAN INST.FOR RES. (72-3719)	25,000
SIMONSEN LABS. (72-3219)	25,025
SIMONSEN LABS. (73-816)	3,402
SIMONSEN LABS. (71-2101)	15,371
SMALL BUSINESS ADMINISTRATION (74-3719)	7,788
SOUTHERN ANIM.FARMS (73-863)	3,466
SOUTHERN ANIM.FARMS (72-2013)	7,700
SOUTHERN RES.INST. (73-3712)	327,250
SPARTAN RES. ANIM. INC.(73-878)	0
SPARTAN RES. ANIM., INC.(73-858)	0
STANFORD RES. INST. (73-3742)	415,675
STARKS,C.P.,INC. (72-3705)	174,665

CONTRACT	DOLLAR LEVEL
STAGE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
TACONIC FARMS (73-912)	2,573
TEXAS INBRED MICE (68-88)	4,541
TEXAS INBRED MICE (74-3711)	70,473
UPJOHN COMPANY (73-3707)	45,037
UPJOHN COMPANY (72-3709)	6,000
UPJOHN COMPANY (74-3753)	197,379
UTAH, UNIV. OF (72-3710)	32,800
VALUE ENGINEERING COMPANY (73-3706)	97,495
VIRGINIA, UNIV.OF (71-2099)	209,000
WARF INST., INC. (67-0747)	7,490
WARF INST., INC. (66-494)	219,909
WARF INST., INC. (74-3707)	81,196
WASH.STATE UNIV. (72-2059)	0
WISCONSIN,BOARD OF REGENTS OF THE UNIVERSITY OF (73-3733)	38,000
WISCONSIN,UNIV.OF (71-2275)	61,853
YALE UNIVERSITY (71-2339)	57,000
TOTAL	7,797,763

ANALYSIS OF CONTRACTS
BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE IIA: DETERMINATION OF ANTI-TUMOR ACTIVITY	
ARS/SPRAGUE-DAWLEY (71-2342)	28,750
ARS/SPRAGUE-DAWLEY (71-2007)	411,079
ARS/SPRAGUE-DAWLEY (70-2044)	29,517
BATTELLE MEM.INST (70-2008)	50,488
BATTELLE MEM.INST. (69-2251)	0
BATTELLE MEM.INST. (71-2182)	0
BELLAIRE ACRES (73-832)	13,608
BELLAIRE ACRES (73-805)	5,544
BRISTOL-MYERS CO. (69-35)	173,790
CHAS. RIV. BREEDING LABS. (73-807)	6,860
CHAS. RIV. BREEDING LABS. (73-839)	17,066
CHAS.RIV.BREEDING LABS. (71-2343)	27,500
CHAS.RIV.BREEDING LABS. (72-2004)	526,714
COLORADO,UNIV.OF (71-2186)	32,178
FLOW RES.ANIM.,INC. (73-838)	14,490
HARLAN INDUSTRIES (73-844)	6,520
HARLAN INDUSTRIES (73-845)	16,017
HARLAN INDUSTRIES (72-3218)	60,750
HAZLETON LABS.,INC. (72-3704)	159,000

CONTRACT	DOLLAR LEVEL
STAGE IIA: DETERMINATION OF ANTI-TUMOR ACTIVITY (CONTINUED)	
HORTON LABS. (73-837)	14,742
IIT RESEARCH INST. (74-3755)	92,846
IIT RESEARCH INST. (68-1021)	550,000
INDIAN CANCER RES. CENTRE (P.L.480)	0
INST.JULES BORDET (72-2053)	10,000
IST. DI RICHERCHE FARM. 'MARIO NEGRI' (73-3720)	127,066
JACKSON LAB. (74-125)	77,251
JAPANESE FD. FOR CANCER RESEARCH (72-2054)	10,932
KANSAS, UNIV.OF (71-2010)	55,514
LAB. SUPPLY CO. (73-840)	16,728
LAB. SUPPLY CO. (73-835)	6,463
LAB.SUPPLY CO. (70-2041)	84,394
LEO GOODWIN INST. FOR CANCER RESEARCH (73-3729)	116,415
LITTLE,ARTHUR D.INC.(73-3727)	578,889
LITTLE,ARTHUR D.INC.(72-3257)	151,794
LITTON BIONETICS, INC.(73-3741)	95,971
MASON RES. INST. (71-2259)	397,375
MIAMI, UNIV. OF (70-2225)	151,018
MICHIGAN,STATE OF, DEPT. OF PUBLIC HEALTH (73-3702)	47,746
MICRO. ASSOC.,INC. (73-3728)	229,064

CONTRACT	DOLLAR LEVEL
STAGE IIA: DETERMINATION OF ANTI-TUMOR ACTIVITY (CONTINUED)	
MIDWEST RES. INST. (73-3743)	20,694
MURPHY BREEDING LABS. (73-868)	5,463
N.Y. STATE DEPT. OF HEALTH (73-3726)	34,125
N.Y. STATE DEPT.OF HEALTH (70-2035)	61,190
NEW JERSEY COLLEGE OF MED.& DENTISTRY (72-3211)	55,125
PARKE, DAVIS & CO. (74-3754)	79,069
PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (71-2188)	4,015
RESEARCH TRIANGLE INST. (69-2019)	6,850
SCRIPPS CLINIC & RES. FD. (72-2042)	12,282
SIMONSEN LABS. (72-3219)	112,604
SIMONSEN LABS. (73-816)	15,309
SIMONSEN LABS. (71-2101)	69,167
SOUTHERN ANIM.FARMS (73-863)	15,592
SOUTHERN ANIM.FARMS (72-2013)	49,500
SOUTHERN RES.INST. (71-2098)	37,846
SPARTAN RES. ANIM. INC. (73-878)	0
SPARTAN RES. ANIM., INC. (73-858)	0
STANFORD RES. INST. (73-3742)	17,319
TACONIC FARMS (73-912)	11,576
TEXAS INBRED MICE (68-88)	20,429

CONTRACT	DOLLAR LEVEL
STAGE IIA: DETERMINATION OF ANTI-TUMOR ACTIVITY (CONTINUED)	
TEXAS INBRED MICE (74-3711)	317,124
UPJOHN COMPANY (73-3707)	23,454
UPJOHN COMPANY (74-3753)	58,866
UTAH, UNIV. OF (72-3710)	7,200
VALUE ENGINEERING COMPANY (73-3706)	417,831
WARF INST., INC. (66-494)	315,765
WASH.STATE UNIV. (72-2059)	0
YALE UNIVERSITY (71-2339)	19,000
YALE UNIVERSITY (73-3711)	16,027
TOTAL	6,197,503

ANALYSIS OF CONTRACTS
BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE IIB: EXPERIMENTAL FORMULATION, DEVELOPMENT	
BEN VENUE LABS.,INC.(74-3701)	54,003
KANSAS, UNIV. OF (72-3217)	90,000
PHILIPS ROXANE LABS.(71-2139)	12,992
TENNESSEE,UNIV.OF (70-2084)	18,750
UPJOHN COMPANY (74-3753)	3,462
TOTAL	179,207

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY CONTRACT	DOLLAR LEVEL
STAGE IIB: DETAILED EVALUATION OF NEW DRUGS	
ALLEGHENY GENERAL HOSPITAL (74-3730)	79,305
ARS/SPRAGUE-DAWLEY (71-2342)	86,250
ARS/SPRAGUE-DAWLEY (71-2007)	216,357
ARS/SPRAGUE-DAWLEY (70-2044)	110,691
ATOMIC ENERGY COMM. (71-56)	24,500
BATTELLE MEM.INST (70-2008)	18,432
BATTELLE MEM.INST. (69-2251)	0
BATTELLE MEM.INST. (71-2182)	0
BELLAIRE ACRES (73-832)	4,968
BELLAIRE ACRES (73-805)	2,024
CATHOLIC MED.CTR OF BROOKLYN & QUEENS, INC.(72-3703)	341,587
CHAS. RIV. BREEDING LABS.(73-807)	2,504
CHAS. RIV. BREEDING LABS.(73-839)	6,230
CHAS.RIV.BREEDING LABS.(71-2343)	82,500
CHAS.RIV.BREEDING LABS. (72-2004)	192,292
CHAS.RIV.BREEDING LABS.(70-2043)	124,200
FLOW RES.ANIM.,INC. (73-838)	5,290
HARLAN INDUSTRIES (73-844)	2,380
HARLAN INDUSTRIES (73-845)	5,847

CONTRACT	DOLLAR LEVEL
STAGE IIB: DETAILED EVALUATION OF NEW DRUGS (CONTINUED)	
HARLAN INDUSTRIES (72-3218)	64,800
HAZLETON LABS.,INC. (72-3704)	68,900
HORTON LABS. (73-837)	5,382
IIT RESEARCH INST. (68-1021)	350,000
INST. OF CANC. RES. ROYAL CANC.HOSPITAL (72-3717)	36,000
JACKSON LAB. (74-125)	104,289
JAPANESE FD. FOR CANCER RESEARCH (72-2054)	10,932
KANSAS, UNIV.OF (71-2010)	20,267
LAB. SUPPLY CO. (73-840)	6,106
LAB. SUPPLY CO. (73-835)	2,359
LAB.SUPPLY CO. (70-2041)	30,810
LEO GOODWIN INST. FOR CANCER RESEARCH (73-3729)	42,500
LITTLE,ARTHUR D.INC.(73-3727)	536,009
LITTLE,ARTHUR D.INC.(72-3711)	91,360
LITTON BIONETICS, INC. (72-3294)	164,065
LITTON BIONETICS, INC. (73-3741)	223,931
LITTON BIONETICS, INC., (72-2062)	36,341
MASON RES. INST. (71-2259)	220,763
MICRO. ASSOC.,INC. (73-3728)	671,921
MURPHY BREEDING LABS. (73-868)	1,994

CONTRACT	DOLLAR LEVEL
STAGE IIB: DETAILED EVALUATION OF NEW DRUGS (CONTINUED)	
N.Y. STATE DEPT.OF HEALTH (70-2035)	22,339
NEW JERSEY COLLEGE OF MED.& DENTISTRY (72-3211)	20,125
NOTRE DAME,UNIV.OF (72-3718)	110,000
PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (71-2188)	9,368
SIMONSEN LABS. (72-3219)	41,109
SIMONSEN LABS. (73-816)	5,589
SIMONSEN LABS. (71-2101)	25,251
SMALL BUSINESS ADMINISTRATION (74-3719)	70,084
SOUTHERN ANIM.FARMS (73-863)	5,692
SOUTHERN ANIM.FARMS (72-2013)	52,804
SOUTHERN RES.INST. (66-29)	82,500
SOUTHERN RES.INST. (71-2098)	88,303
SPARTAN RES. ANIM. INC.(73-878)	0
SPARTAN RES. ANIM., INC.(73-858)	0
TACONIC FARMS (73-912)	4,226
TEXAS INBRED MICE (68-88)	7,458
TEXAS INBRED MICE (74-3711)	115,775
TULANE UNIVERSITY (73-3701)	123,491
UPJOHN COMPANY (73-3707)	9,382
UPJOHN COMPANY (74-3753)	34,627

CONTRACT		DOLLAR LEVEL
STAGE IIB: DETAILED EVALUATION OF NEW DRUGS	(CONTINUED)	
VALUE ENGINEERING COMPANY (73-3706)		181,060
WARF INST., INC. (66-494)		28,193
WASH.STATE UNIV. (72-2059)		0
TOTAL		5,031,458

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE IIB: PROC. OF AGENTS FOR PRE-CLIN. STUDIES	
ABBOTT LABORATORIES (74-3704)	130,848
AEROJET-GEN.CORP. (68-1300)	38,000
ALDRICH CHEMICAL CO. (72-3706)	122,500
DOW CHEMICAL CO. (71-2165)	60,000
MERCK & COMPANY (72-2002)	387,516
MIDWEST RES. INST. (73-3722)	42,877
MONSANTO RES.CORP. (73-3709)	26,724
MONSANTO RES.CORP. (72-3715)	168,000
PARKE,DAVIS & CO. (72-3708)	133,500
PHARM-ECO (72-3707)	64,000
STANFORD RES.INST. (73-3723)	68,488
STANFORD RES.INST. (71-2162)	160,000
STARKS ASSOC., INC. (72-3203)	203,438
TOTAL	1,605,891

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE III: TOXICOLOGY & PHARM. IN LARGE ANIMALS	
ASSOCIATED BIOMEDIC SYSTEMS, INC. (70-2271)	32,078
BATTELLE MEMORIAL INSTITUTE (74-3746)	1,773,947
COLORADO,UNIV.OF (71-2186)	75,081
DOW CHEMICAL CO. (72-3712)	109,810
HAZLETON RES.ANIM., INC. (73-879)	1,272
IIT RESEARCH INST. (67-1141)	0
IST. DI RICHERCHE FARM. "MARIO NEGRI" (72-3242)	29,000
JOHNS HOPKINS UNIV. (74-3718)	43,957
LITTLE,ARTHUR D.INC.(73-3727)	879,054
LITTON BIONETICS, INC.,(72-2062)	72,683
LITTON BIONETICS, INC.,(71-2340)	467,085
MARSHALL RES.ANIM., INC.(73-889)	0
MASON RES.INST. (70-2055)	151,693
MICHIGAN,REGENTS OF THE UNIVERSITY OF (72-3714)	0
MICRO. ASSOC.,INC. (73-3728)	229,063
N.Y. STATE DEPT. OF HEALTH(73-3726)	63,375
SOUTH SHORE ANALYT. & RES.LAB.(64-932)	0
SOUTHERN RES.INST. (65-654)	80,506
SOUTHERN RES.INST. (70-2083)	135,334

CONTRACT	DOLLAR LEVEL
STAGE III: TOXICOLOGY & PHARM. IN LARGE ANIMALS (CONTINUED)	
TEXAS,UNIV.OF (66-1156)	246,998
TRW, INC. (71-2190)	30,000
TULANE UNIVERSITY (73-3701)	6,499
WISCONSIN,UNIV.OF (71-2275)	61,852
YALE UNIVERSITY (73-3740)	0
TOTAL	4,489,287

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE III: PRODUCT. & FORM. FOR CLINICAL TRIALS	
AEROJET-GEN.CORP. (68-1300)	152,000
ALDRICH CHEMICAL CO.(72-3706)	52,500
BEN VENUE LABS.,INC. (74-3701)	486,020
DOW CHEMICAL CO. (71-2165)	140,000
MERCK & COMPANY (72-2002)	129,172
MIDWEST RES. INST. (73-3722)	171,504
MONSANTO RES.CORP. (73-3709)	240,513
MONSANTO RES.CORP. (72-3715)	42,000
PARKE,DAVIS & CO. (72-3708)	133,500
PHARM-ECO (72-3707)	96,000
PHILIPS ROXANE LABS.(71-2139)	51,967
STANFORD RES.INST. (73-3723)	273,948
STANFORD RES.INST. (71-2162)	40,000
STARKS ASSOC., INC. (72-3203)	135,624
TENNESSEE,UNIV.OF (70-2084)	56,250
TRACOR JITCO, INC. (73-3744)	0
TOTAL	2,200,998

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE IV: CLINICAL TRIALS - PHASE I	
ATOMIC ENERGY COMM. (71-59)	0
DUKE UNIVERSITY MEDICAL CENTER (72-2044)	82,300
GEORGETOWN UNIV. (71-2123)	57,474
HAZLETON LABS.,INC. (73-3705)	68,480
JAPANESE FD. FOR CANCER RESEARCH (72-2054)	16,398
MARYLAND,UNIV.OF (74-3748)	382,950
YALE UNIVERSITY (73-3711)	3,827
TOTAL	611,429

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE IV: INITIAL CLIN. PHARM. STUDIES IN MAN	
CHICAGO, UNIV. OF (73-3748)	27,000
JEFFERSON MED.COLL. (70-2136)	52,200
MARYLAND,UNIV.OF (74-3748)	255,300
TEXAS,UNIV.OF (66-1156)	49,399
UPJOHN COMPANY (73-3707)	15,949
UPJOHN COMPANY (74-3753)	51,941
WALTER REED ARMY MED.CENTER (64-5)	1,032
YALE UNIVERSITY (73-3711)	4,066
TOTAL	456,887

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE V: CLINICAL TRIALS - PHASE II	
ALBANY MED.COLLEGE (72-2074)	17,185
ARIZONA BOARD OF REGENTS UNIV. OF ARIZONA (73-3713)	123,000
ATOMIC ENERGY COMM. (71-60)	85,000
AUTOMATION INDUST., INC.(73-3717)	17,815
BOWMAN GRAY SCHOOL OF MED.(68-950)	5,046
CALIFORNIA#, UNIV.OF (74-3706)	9,120
CONNECTICUT, UNIV. OF (71-2181)	10,687
CONTROL DATA CORP. (73-3739)	10,800
DUKE UNIVERSITY (70-2030)	16,235
GEORGETOWN UNIV. (73-3745)	28,000
GEORGETOWN UNIV. (71-2123)	57,472
HAZLETON LABS.,INC. (73-3705)	68,480
HAZLETON LABS.,INC. (73-3708)	174,250
INDIANA UNIV.FDN. (70-2028)	8,973
IST. NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (73-3714)	31,750
KENTUCKY, UNIV.OF (67-1173)	5,838
LITTLE, ARTHUR D.INC.(72-3711)	91,360
MAKERERE UNIV. COLLEGE COUNCIL (67-1343)	71,246
MARYLAND, UNIV.OF (74-3748)	382,950

CONTRACT	DOLLAR LEVEL
STAGE V: CLINICAL TRIALS - PHASE II (CONTINUED)	
MAYO FOUNDATION (71-2185)	127,000
MAYO FOUNDATION (70-2066)	88,000
MAYO FOUNDATION (73-3735)	25,294
MEMORIAL HOSPITAL FOR CANCER & ALLIED DISEASES (72-2073)	0
MISSOURI,CURATORS OF THE UNIVERSITY OF(71-2323)	138,434
MOUNT SINAI SCHOOL OF MEDICINE (74-3703)	24,939
N.Y. STATE DEPT.OF HEALTH (72-2072)	18,900
NEW YORK UNIV. (67-1174)	9,890
OHIO STATE UNIV. (67-1175)	7,273
RESEARCH FD. OF STATE UNIV. OF NEW YORK (73-3715)	22,790
TECHNICON INSTRUMENTS CORP. (73-3746)	0
TEXAS, UNIV. OF (73-3737)	0
TEXAS, UNIV. OF (73-3710)	133,000
UNIV. HOSPITAL (73-3725)	0
VERMONT, UNIV. OF (73-3716)	13,465
VETERANS ADMIN. (64-4C)	126,400
WALTER REED ARMY MED.CENTER(64-5)	23,701
TOTAL	1,974,288

ANALYSIS OF CONTRACTS
BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE VI: CLINICAL TRIALS - PHASE III	
ABCOR, INC. (70-2239)	3,500
ALBANY MED.COLLEGE (72-2074)	17,185
ATOMIC ENERGY COMM. (74-0101)	100,000
AUTOMATION INDUST., INC.(73-3717)	44,535
BOWMAN GRAY SCHOOL OF MED.(68-950)	11,773
CALIFORNIA,UNIV.OF (70-2092)	414,000
CALIFORNIA#,UNIV.OF (74-3706)	21,278
CAPETOWN, UNIV. OF (73-3738)	12,000
CONNECTICUT, UNIV. OF (71-2181)	24,922
CONTROL DATA CORP. (73-3739)	25,200
DUKE UNIVERSITY (70-2030)	37,879
GEORGETOWN UNIV. (73-3745)	56,000
GEORGETOWN UNIV. (71-2123)	86,208
HAZLETON LABS.,INC. (73-3705)	102,720
HAZLETON LABS.,INC. (72-3701)	22,400
HEBREW UNIV. (71-2127)	105,000
INDIANA UNIV.FDN. (70-2028)	20,936
IST. NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (73-3714)	15,875
KENTUCKY,UNIV.OF (67-1173)	13,619

CONTRACT	DOLLAR LEVEL
STAGE VI: CLINICAL TRIALS - PHASE III (CONTINUED)	
LITTLE,ARTHUR D.INC.(72-3711)	205,560
MAKERERE UNIV. COLLEGE COUNCIL (67-1343)	106,868
MARYLAND,UNIV.OF (74-3748)	382,950
MAYO FOUNDATION (73-3735)	37,939
MEMORIAL HOSPITAL FOR CANCER & ALLIED DISEASES (72-2073)	0
MOUNT SINAI SCHOOL OF MEDICINE (74-3703)	24,939
N.Y. STATE DEPT. OF HEALTH (73-3734)	66,048
N.Y. STATE DEPT.OF HEALTH (72-2072)	18,900
NEW YORK UNIV. (67-1174)	23,075
OHIO STATE UNIV. (67-1175)	16,968
OHIO STATE UNIV. (73-3736)	56,800
RESEARCH FD. OF STATE UNIV. OF NEW YORK (73-3715)	56,975
TEXAS, UNIV. OF (73-3737)	0
TEXAS, UNIV. OF (73-3710)	57,000
TEXAS,UNIV.OF (71-2094)	253,814
VERMONT, UNIV. OF (73-3716)	13,462
VETERANS ADMIN. (64-4C)	379,200
WALTER REED ARMY MED.CENTER(64-5)	14,941
TOTAL	2,850,469

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE VI: TOX. & PHARM. STUDY LARGE ANIM. & MAN	
BRITISH COLUMBIA, UNIV. OF (72-3223)	16,800
COLORADO STATE UNIV. (71-2192)	75,750
GEORGE WASH. UNIV. (71-2206)	68,987
HAZLETON LABS.,INC. (73-3708)	174,250
HAZLETON LABS.,INC. (74-3702)	311,235
IST. DI RICHERCHE FARM. "MARIO NEGRI" (72-3242)	29,000
JEFFERSON MED.COLL. (70-2136)	5,800
KANSAS,UNIV.OF (71-2184)	100,000
LITTON BIONETICS, INC.,(71-2340)	1,167,714
MARYLAND,UNIV.OF (74-3748)	255,300
MICRO. ASSOC. INC.(69-2177)	70,000
OREGON STATE UNIV. (72-3201)	73,271
SOUTHERN CALIF. UNIVERSITY OF (72-3241)	128,100
SOUTHERN RES.INST. (65-654)	14,206
SOUTHERN RES.INST. (70-2083)	202,999
STANFORD RES.INST. (72-3200)	73,010
TEXAS,UNIV.OF (66-1156)	197,596
WALTER REED ARMY MED.CENTER(64-5)	1,030
WASHINGTON HOSPITAL CENTER	1,030

CONTRACT	DOLLAR LEVEL
STAGE VI: TOX. & PHARM. STUDY LARGE ANIM. & MAN(CONTINUED)	
WASHINGTON,UNIV.OF (71-2193)	0
TOTAL	2,991,933

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE VII: CLINICAL TRIALS - ADJUVANT STUDIES	
AUTOMATION INDUST., INC. (73-3717)	26,721
CAPETOWN, UNIV. OF (73-3738)	18,000
CLEVELAND CLIN. FD. (73-3704)	38,364
GEORGETOWN UNIV. (73-3745)	35,000
GEORGETOWN UNIV. (71-2123)	86,208
HAZLETON LABS., INC. (73-3705)	102,720
HAZLETON LABS., INC. (72-3701)	89,600
IST. NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (73-3714)	15,875
MARYLAND, UNIV. OF (74-3748)	765,900
MAYO FOUNDATION (73-3735)	63,232
N.Y. STATE DEPT OF HEALTH (73-3734)	99,070
OHIO STATE UNIV. (73-3736)	85,200
RESEARCH FD. OF STATE UNIV. OF NEW YORK (73-3715)	79,765
TEXAS, UNIV. OF (73-3737)	0
VETERANS ADMIN. (64-4C)	632,000
WALTER REED ARMY MED. CENTER (64-5)	10,320
TOTAL	2,148,475

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE VIII: UNASSIGNED-ADMIN. & INFORM. FUNCTION	
AUTOMATION INDUST., INC. (73-3732)	224,800
GEORGETOWN UNIV. (73-3745)	21,000
HERNER INFORMATION SERVICES, INC. (74-3720)	162,919
HERNER INFORMATION SERVICES, INC. (72-3244)	0
INST.JULES BORDET (72-2053)	18,000
JAPANESE FD. FOR CANCER RESEARCH (72-2054)	10,932
LITTLE,ARTHUR D.INC.(72-3711)	22,840
LITTON BIONETICS, INC. (72-3294)	129,525
MARYLAND,UNIV.OF (74-3748)	127,650
MILES LABORATORIES (74-3709)	0
NATIONAL ACADEMY OF SCIENCES #16(64-44)	0
NATIONAL ACADEMY OF SCIENCES #6(64-44)	17,500
RESEARCH FD. OF STATE UNIV. OF NEW YORK (73-3715)	68,370
VETERANS ADMIN. (64-4C)	126,400
TOTAL	929,936

TABLE III

ANALYSIS OF DIVISION OF CANCER TREATMENT

ACTIVITIES BY CONTRACT

FOR FISCAL YEAR 1974
AS OF MARCH 15, 1974

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
ABBOTT LABORATORIES (74-3704)				$ 130,848
2B4	PROC. OF PRECLINICAL MATERIALS	- 100% -		
F	OTHER	100%	130,848	
ABCOR, INC. (70-2239)				3,500
6X1	CLINICAL TRIALS - PHASE III	- 100% -		
P	BLOOD PRODUCTS	100%	3,500	
AEROJET-GEN.CORP. (68-1300)				190,000
2B4	PROC. OF PRECLINICAL MATERIALS	- 20% -	38,000	
A	SYNTHETICS PROCUREMENT	20%	38,000	
3X2	DRUG PROD. & FORM FOR CLIN. TRIALS	- 80% -	152,000	
A	SYNTHETICS PRODUCTION	80%	152,000	
AGRICULT.,DEPT.OF (64-1)				349,000
1XX	ACQUISITION OF MATERIALS	- 100% -		
B	PLANT PRODUCTS PROCUREMENT	100%	349,000	
ALBANY MED.COLLEGE (72-2074)				34,370
5XX	CLINICAL TRIALS - PHASE II	- 50% -	17,185	
A	BREAST	50%	17,185	
6X1	CLINICAL TRIALS - PHASE III	- 50% -	17,185	
A	BREAST	50%	17,185	
ALDRICH CHEMICAL CO.(72-3706)				175,000
2B4	PROC. OF PRECLINICAL MATERIALS	- 70% -	122,500	
A	SYNTHETICS PROCUREMENT	70%	122,500	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 30% -	52,500	
A	SYNTHETICS PRODUCTION	30%	52,500	
ALLEGHENY GENERAL HOSPITAL (74-3730)				79,305
2B2	VERIFICATION SCREEN	- 100% -		
A	DETAILED DRUG EVALUATION IN VITRO	50%	39,653	
B	DETAILED DRUG EVALUATION IN VIVO	50%	39,653	
ARIZONA BOARD OF REGENTS ARIZONA STATE UNIV. (71-2308)				48,600
1XX	ACQUISITION OF MATERIALS	- 100% -		
D	MARINE/ARTHROPODS PROCUREMENT	100%	48,600	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
ARIZONA BOARD OF REGENTS UNIV. OF ARIZONA (73-3713)		$	123,000
5XX CLINICAL TRIALS - PHASE II	- 100% -		
Q MARKERS	50%	61,500	
R DATA PROCESSING AND SUPPORT	50%	61,500	
ARIZONA BOARD OF REGENTS UNIV. OF ARIZONA (73-3750)			114,500
1XX ACQUISITION OF MATERIALS	- 100% -		
B PLANT PRODUCTS PROCUREMENT	79%	90,455	
C FERMENTATION/ANTIBIOTICS PROCUREMENT	10%	11,450	
E BIOASSAY OF NATURAL PRODUCTS	11%	12,595	
ARS/SPRAGUE-DAWLEY (71-2342)			115,000
2AX BASIC SCREEN	- 25% -	28,750	
D ANIMALS	25%	28,750	
2B2 VERIFICATION SCREEN	- 75% -	86,250	
D RELATED NEW ASSAY DEVELOPMENT	10%	11,500	
E ANIMALS	65%	74,750	
ARS/SPRAGUE-DAWLEY (71-2007)			721,192
1XX ACQUISITION OF MATERIALS	- 13% -	93,756	
E BIOASSAY OF NATURAL PRODUCTS	6%	43,272	
F ANIMALS	7%	50,483	
2AX BASIC SCREEN	- 57% -	411,079	
C RELATED NEW ASSAY DEVELOPMENT	5%	36,060	
D ANIMALS	52%	375,020	
2B2 VERIFICATION SCREEN	- 30% -	216,357	
D RELATED NEW ASSAY DEVELOPMENT	5%	36,060	
E ANIMALS	25%	180,298	
ARS/SPRAGUE-DAWLEY (70-2044)			147,589
1XX ACQUISITION OF MATERIALS	- 5% -	7,381	
F ANIMALS	5%	7,379	
2AX BASIC SCREEN	- 20% -	29,517	
D ANIMALS	20%	29,518	
2B2 VERIFICATION SCREEN	- 75% -	110,691	
E ANIMALS	75%	110,692	
ASH STEVENS,INC. (71-2314)			2,231
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	2,231	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
ASSOCIATED BIOMEDIC SYSTEMS, INC. (70-2271)			$ 160,390
1XX ACQUISITION OF MATERIALS	- 80% -	128,312	
H OTHER	80%	128,312	
3X1 PHARMACOLOGY/TOXICOLOGY	- 20% -	32,078	
F PHARMACOLOGY	20%	32,078	
ATOMIC ENERGY COMM. (71-60)			85,000
5XX CLINICAL TRIALS - PHASE II	- 100% -		
Q MARKERS	90%	76,500	
R DATA PROCESSING AND SUPPORT	10%	8,500	
ATOMIC ENERGY COMM. (71-59)			0
4X1 CLINICAL TRIALS - PHASE I	- 100% -		
D OTHER	100%		
ATOMIC ENERGY COMM. (71-56)			24,500
2B2 VERIFICATION SCREEN	- 100% -		
A DETAILED DRUG EVALUATION IN VITRO	70%	17,150	
D RELATED NEW ASSAY DEVELOPMENT	30%	7,350	
ATOMIC ENERGY COMM. (74-0101)			100,000
6X1 CLINICAL TRIALS - PHASE III	- 100% -		
P BLOOD PRODUCTS	100%	100,000	
AUTOMATION INDUST., INC.(73-3717)			89,071
5XX CLINICAL TRIALS - PHASE II	- 20% -	17,815	
R DATA PROCESSING AND SUPPORT	20%	17,814	
6X1 CLINICAL TRIALS - PHASE III	- 50% -	44,535	
S DATA PROCESSING AND SUPPORT	50%	44,536	
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 30% -	26,721	
P DATA PROCESSING AND SUPPORT	30%	26,721	
AUTOMATION INDUST., INC. (73-3732)			224,800
8XX PROGRAM MANAGEMENT	- 100% -		
B DISSEMINATION OF INFORMATION	92%	206,816	
C DATA PROCESSING AND SUPPORT	8%	17,984	
BATTELLE MEM.INST (70-2008)			80,140
1XX ACQUISITION OF MATERIALS	- 14% -	11,220	
E BIOASSAY OF NATURAL PRODUCTS	2%	1,603	
F ANIMALS	12%	9,617	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2AX BASIC SCREEN	- 63% -	50,488	
C RELATED NEW ASSAY DEVELOPMENT	3%	2,404	
D ANIMALS	60%	48,084	
2B2 VERIFICATION SCREEN	- 23% -	18,432	
D RELATED NEW ASSAY DEVELOPMENT	3%	2,404	
E ANIMALS	20%	16,028	
BATTELLE MEM.INST. (69-2251)			$ 0
1XX ACQUISITION OF MATERIALS	- 14% -		
E BIOASSAY OF NATURAL PRODUCTS	7%		
F ANIMALS	7%		
2AX BASIC SCREEN	- 63% -		
C RELATED NEW ASSAY DEVELOPMENT	5%		
D ANIMALS	58%		
2B2 VERIFICATION SCREEN	- 23% -		
D RELATED NEW ASSAY DEVELOPMENT	3%		
E ANIMALS	20%		
BATTELLE MEM.INST. (71-2182)			0
1XX ACQUISITION OF MATERIALS	- 1% -		
E BIOASSAY OF NATURAL PRODUCTS	1%		
2AX BASIC SCREEN	- 79% -		
B PRIMARY SCREENING IN VIVO	79%		
2B2 VERIFICATION SCREEN	- 20% -		
B DETAILED DRUG EVALUATION IN VIVO	20%		
BATTELLE MEMORIAL INSTITUTE (74-3746)			1,773,947
3X1 PHARMACOLOGY/TOXICOLOGY	- 100% -		
A PROTOCOL TOXICITY STUDIES	70%	1,241,763	
B SPECIAL TOXICITY STUDIES	14%	248,353	
C ANIMALS	1%	17,739	
D DATA PROCESSING AND SUPPORT	15%	266,092	
BELLAIRE ACRES (73-832)			21,600
1XX ACQUISITION OF MATERIALS	- 14% -	3,024	
E BIOASSAY OF NATURAL PRODUCTS	2%	432	
F ANIMALS	12%	2,592	
2AX BASIC SCREEN	- 63% -	13,608	
C RELATED NEW ASSAY DEVELOPMENT	3%	648	
D ANIMALS	60%	12,960	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
2B2	VERIFICATION SCREEN	- 23% -	4,968	
E	ANIMALS	23%	4,968	
BELLAIRE ACRES (73-805)				$ 8,800
1XX	ACQUISITION OF MATERIALS	- 14% -	1,232	
E	BIOASSAY OF NATURAL PRODUCTS	2%	176	
F	ANIMALS	12%	1,056	
2AX	BASIC SCREEN	- 63% -	5,544	
C	RELATED NEW ASSAY DEVELOPMENT	3%	264	
D	ANIMALS	60%	5,280	
2B2	VERIFICATION SCREEN	- 23% -	2,024	
D	RELATED NEW ASSAY DEVELOPMENT	3%	264	
E	ANIMALS	20%	1,760	
BEN VENUE LABS., INC. (74-3701)				540,023
2B1	FORMULATION	- 10% -	54,003	
A	DEVEL. OF EXPERIMENTAL FORMULATIONS	10%	54,002	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 90% -	486,020	
F	FORMULATION	70%	378,016	
G	ANALYTICAL AND QUALITY CONTROL	20%	108,005	
BOWMAN GRAY SCHOOL OF MED. (68-950)				16,819
5XX	CLINICAL TRIALS - PHASE II	- 30% -	5,046	
J	BRAIN	30%	5,046	
6X1	CLINICAL TRIALS - PHASE III	- 70% -	11,773	
J	BRAIN	70%	11,773	
BRISTOL-MYERS CO. (69-35)				457,354
1XX	ACQUISITION OF MATERIALS	- 62% -	283,560	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	62%	283,559	
2AX	BASIC SCREEN	- 38% -	173,794	
A	PRIMARY SCREENING IN VITRO	10%	45,735	
B	PRIMARY SCREENING IN VIVO	28%	128,059	
BRITISH COLUMBIA, UNIV. OF (72-3223)				84,000
1XX	ACQUISITION OF MATERIALS	- 80% -	67,200	
A	SYNTHETICS PROCUREMENT	80%	67,200	
6X2	TOXICOLOGY/PHARMACOLOGY	- 20% -	16,800	
D	OTHER	20%	16,800	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
CALIFORNIA,UNIV.OF (70-2092)			$ 414,000
6X1 CLINICAL TRIALS - PHASE III	- 100% -		
Q TRANSFUSION RESEARCH	100%	414,000	
CALIFORNIA#,UNIV.OF (74-3706)			30,398
5XX CLINICAL TRIALS - PHASE II	- 30% -	9,120	
J BRAIN	30%	9,119	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	21,278	
J BRAIN	70%	21,279	
CAPETOWN, UNIV. OF (73-3738)			30,000
6X1 CLINICAL TRIALS - PHASE III	- 40% -	12,000	
C LUNG	40%	12,000	
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 60% -	18,000	
C LUNG	60%	18,000	
CATHOLIC MED.CTR OF BROOKLYN & QUEENS, INC.(72-3703)			341,587
2B2 VERIFICATION SCREEN	- 100% -		
B DETAILED DRUG EVALUATION IN VIVO	90%	307,428	
D RELATED NEW ASSAY DEVELOPMENT	10%	34,159	
CHAS. RIV. BREEDING LABS.(73-807)			10,890
1XX ACQUISITION OF MATERIALS	- 14% -	1,526	
E BIOASSAY OF NATURAL PRODUCTS	2%	218	
F ANIMALS	12%	1,307	
2AX BASIC SCREEN	- 63% -	6,860	
C RELATED NEW ASSAY DEVELOPMENT	3%	327	
D ANIMALS	60%	6,534	
2B2 VERIFICATION SCREEN	- 23% -	2,504	
D RELATED NEW ASSAY DEVELOPMENT	3%	327	
E ANIMALS	20%	2,178	
CHAS. RIV. BREEDING LABS.(73-839)			27,090
1XX ACQUISITION OF MATERIALS	- 14% -	3,794	
E BIOASSAY OF NATURAL PRODUCTS	2%	542	
F ANIMALS	12%	3,251	
2AX BASIC SCREEN	- 63% -	17,066	
C RELATED NEW ASSAY DEVELOPMENT	3%	813	
D ANIMALS	60%	16,254	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2B2 VERIFICATION SCREEN	- 23% -	6,230	
E ANIMALS	23%	6,231	
CHAS.RIV.BREEDING LABS.(71-2343)		$	110,000
2AX BASIC SCREEN	- 25% -	27,500	
C RELATED NEW ASSAY DEVELOPMENT	5%	5,500	
D ANIMALS	20%	22,000	
2B2 VERIFICATION SCREEN	- 75% -	82,500	
D RELATED NEW ASSAY DEVELOPMENT	10%	11,000	
E ANIMALS	65%	71,500	
CHAS.RIV.BREEDING LABS.(72-2004)			836,055
1XX ACQUISITION OF MATERIALS	- 14% -	117,049	
E BIOASSAY OF NATURAL PRODUCTS	2%	16,721	
F ANIMALS	12%	100,327	
2AX BASIC SCREEN	- 63% -	526,714	
C RELATED NEW ASSAY DEVELOPMENT	3%	25,082	
D ANIMALS	60%	501,633	
2B2 VERIFICATION SCREEN	- 23% -	192,292	
E ANIMALS	23%	192,293	
CHAS.RIV.BREEDING LABS.(70-2043)			124,200
2B2 VERIFICATION SCREEN	- 100% -		
B DETAILED DRUG EVALUATION IN VIVO	5%	6,210	
D RELATED NEW ASSAY DEVELOPMENT	5%	6,210	
E ANIMALS	90%	111,780	
CHEM.ABSTR.SERVICE (65-1021)			350,952
1XX ACQUISITION OF MATERIALS	- 100% -		
G DATA PROCESSING AND SUPPORT	100%	350,952	
CHICAGO, UNIV. OF (73-3748)			27,000
4X2 CLINICAL PHARMACOLOGY	- 100% -		
A CLIN. PHARMACOLOGY & PHARMACOKINETICS	100%	27,000	
CLEVELAND CLIN. FD. (73-3704)			38,364
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
Q OTHER	100%	38,364	
COLLABORATIVE RES., INC.(66-506)			89,557
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	89,557	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
COLLABORATIVE RES., INC. (71-2326)			$ 51,000
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	51,000	
COLORADO STATE UNIV.(71-2192)			75,750
6X2 TOXICOLOGY/PHARMACOLOGY	- 100% -		
A ANIMAL STUDIES	100%	75,750	
COLORADO,UNIV.OF (71-2186)			107,259
2AX BASIC SCREEN	- 30% -	32,178	
A PRIMARY SCREENING IN VITRO	30%	32,178	
3X1 PHARMACOLOGY/TOXICOLOGY	- 70% -	75,081	
F PHARMACOLOGY	70%	75,081	
CONNECTICUT, UNIV. OF (71-2181)			35,604
5XX CLINICAL TRIALS - PHASE II	- 30% -	10,682	
J BRAIN	30%	10,681	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	24,922	
J BRAIN	70%	24,923	
CONTROL DATA CORP. (73-3739)			36,000
5XX CLINICAL TRIALS - PHASE II	- 30% -	10,800	
R DATA PROCESSING AND SUPPORT	30%	10,800	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	25,200	
S DATA PROCESSING AND SUPPORT	70%	25,200	
DELAWARE, UNIV.OF (69-2115)			0
1XX ACQUISITION OF MATERIALS	- 100% -		
C FERMENTATION/ANTIBIOTICS PROCUREMENT	100%		
DOW CHEMICAL CO. (73-3724)			160,000
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	160,000	
DOW CHEMICAL CO. (71-2165)			200,000
2B4 PROC. OF PRECLINICAL MATERIALS	- 30% -	60,000	
A SYNTHETICS PROCUREMENT	30%	60,000	
3X2 DRUG PROD. & FORM. FOR CLIN. TRIALS	- 70% -	140,000	
A SYNTHETICS PRODUCTION	70%	140,000	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
DOW CHEMICAL CO. (71-2313)			$ 0
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%		
DOW CHEMICAL CO. (72-3712)			109,810
3X1 PHARMACOLOGY/TOXICOLOGY	- 100% -		
B SPECIAL TOXICITY STUDIES	100%	109,810	
DUKE UNIVERSITY (70-2030)			54,114
5XX CLINICAL TRIALS - PHASE II	- 30% -	16,235	
J BRAIN	30%	16,234	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	37,879	
J BRAIN	70%	37,880	
DUKE UNIVERSITY MEDICAL CENTER (72-2044)			82,300
4X1 CLINICAL TRIALS - PHASE I	- 100% -		
A CELL KINETICS	100%	82,300	
FLOW RES.ANIM.,INC. (73-338)			23,000
1XX ACQUISITION OF MATERIALS	- 14% -	3,220	
E BIOASSAY OF NATURAL PRODUCTS	2%	460	
F ANIMALS	12%	2,760	
2AX BASIC SCREEN	- 63% -	14,490	
C RELATED NEW ASSAY DEVELOPMENT	3%	690	
D ANIMALS	60%	13,800	
2B2 VERIFICATION SCREEN	- 23% -	5,290	
C MOLECULAR BIOLOGY PROGRAM	3%	690	
D RELATED NEW ASSAY DEVELOPMENT	20%	4,600	
GEORGE WASH. UNIV. (71-2206)			68,987
6X2 TOXICOLOGY/PHARMACOLOGY	- 100% -		
A ANIMAL STUDIES	95%	65,538	
C DATA PROCESSING AND SUPPORT	5%	3,449	
GEORGETOWN UNIV. (73-3745)			140,000
5XX CLINICAL TRIALS - PHASE II	- 20% -	28,000	
R DATA PROCESSING AND SUPPORT	20%	28,000	
6X1 CLINICAL TRIALS - PHASE III	- 40% -	56,000	
S DATA PROCESSING AND SUPPORT	40%	56,000	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 25% -	35,000	
P	DATA PROCESSING AND SUPPORT	25%	35,000	
8XX	PROGRAM MANAGEMENT	- 15% -	21,000	
A	ADMINISTRATION	5%	7,000	
B	DISSEMINATION OF INFORMATION	5%	7,000	
C	DATA PROCESSING AND SUPPORT	5%	7,000	
GEORGETOWN UNIV. (71-2123)				$ 287,362
4X1	CLINICAL TRIALS - PHASE I	- 20% -	57,474	
B	PHARMACOLOGY/TOXICOLOGY	20%	57,472	
5XX	CLINICAL TRIALS - PHASE II	- 20% -	57,472	
G	TESTICULAR	3%	8,621	
J	BRAIN	4%	11,494	
K	LYMPHOMAS	5%	14,368	
L	LEUKEMIA	4%	11,494	
N	OTHER DISEASES	4%	11,494	
6X1	CLINICAL TRIALS - PHASE III	- 30% -	86,208	
G	TESTICULAR	4%	11,494	
J	BRAIN	4%	11,494	
K	LYMPHOMAS	8%	22,989	
L	LEUKEMIA	8%	22,989	
N	OTHER DISEASES	6%	17,242	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 30% -	86,208	
G	TESTICULAR	3%	8,621	
J	BRAIN	4%	11,494	
K	LYMPHOMAS	8%	22,989	
L	LEUKEMIA	8%	22,989	
N	OTHER DISEASES	7%	20,115	
GEORGIA, UNIV. OF (71-2311)				0
1XX	ACQUISITION OF MATERIALS	- 100% -		
A	SYNTHETICS PROCUREMENT	100%		
HARLAN INDUSTRIES (73-844)				10,350
1XX	ACQUISITION OF MATERIALS	- 14% -	1,450	
E	BIOASSAY OF NATURAL PRODUCTS	2%	207	
F	ANIMALS	12%	1,242	
2AX	BASIC SCREEN	- 63% -	6,520	
C	RELATED NEW ASSAY DEVELOPMENT	3%	311	
D	ANIMALS	60%	6,210	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
2B2	VERIFICATION SCREEN	- 23% -	2,380	
D	RELATED NEW ASSAY DEVELOPMENT	3%	311	
E	ANIMALS	20%	2,070	
HARLAN INDUSTRIES (73-845)			$	25,425
1XX	ACQUISITION OF MATERIALS	- 14% -	3,561	
E	BIOASSAY OF NATURAL PRODUCTS	2%	509	
F	ANIMALS	12%	3,051	
2AX	BASIC SCREEN	- 63% -	16,017	
C	RELATED NEW ASSAY DEVELOPMENT	3%	763	
D	ANIMALS	60%	15,255	
2B2	VERIFICATION SCREEN	- 23% -	5,847	
E	ANIMALS	23%	5,848	
HARLAN INDUSTRIES (72-3218)				135,000
1XX	ACQUISITION OF MATERIALS	- 7% -	9,450	
F	ANIMALS	7%	9,450	
2AX	BASIC SCREEN	- 45% -	60,750	
C	RELATED NEW ASSAY DEVELOPMENT	3%	4,050	
D	ANIMALS	42%	56,700	
2B2	VERIFICATION SCREEN	- 48% -	64,800	
D	RELATED NEW ASSAY DEVELOPMENT	5%	6,750	
E	ANIMALS	43%	58,050	
HAWAII, UNIV. OF (73-3747)				65,000
1XX	ACQUISITION OF MATERIALS	- 100% -		
B	PLANT PRODUCTS PROCUREMENT	100%	65,000	
HAZLETON LABS.,INC. (73-3705)				342,400
4X1	CLINICAL TRIALS - PHASE I	- 20% -	68,480	
B	PHARMACOLOGY/TOXICOLOGY	20%	68,480	
5XX	CLINICAL TRIALS - PHASE II	- 20% -	68,480	
G	TESTICULAR	3%	10,272	
J	BRAIN	4%	13,696	
K	LYMPHOMAS	5%	17,120	
L	LEUKEMIA	4%	13,696	
N	OTHER DISEASES	4%	13,696	
6X1	CLINICAL TRIALS - PHASE III	- 30% -	102,720	
G	TESTICULAR	4%	13,696	
J	BRAIN	4%	13,696	
K	LYMPHOMAS	8%	27,392	
L	LEUKEMIA	8%	27,392	
N	OTHER DISEASES	6%	20,544	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 30% -	102,720	
G	TESTICULAR	3%	10,272	
J	BRAIN	4%	13,696	
K	LYMPHOMAS	8%	27,392	
L	LEUKEMIA	8%	27,392	
M	OTHER DISEASES	7%	23,968	
HAZLETON LABS.,INC. (73-3708)				$ 348,500
5XX	CLINICAL TRIALS - PHASE II	- 50% -	174,250	
Q	MARKERS	50%	174,250	
6X2	TOXICOLOGY/PHARMACOLOGY	- 50% -	174,250	
A	ANIMAL STUDIES	50%	174,250	
HAZLETON LABS.,INC. (72-3704)				265,000
1XX	ACQUISITION OF MATERIALS	- 14% -	37,100	
E	BIOASSAY OF NATURAL PRODUCTS	14%	37,100	
2AX	BASIC SCREEN	- 60% -	159,000	
B	PRIMARY SCREENING IN VIVO	60%	159,000	
2B2	VERIFICATION SCREEN	- 26% -	68,900	
B	DETAILED DRUG EVALUATION IN VIVO	26%	68,900	
HAZLETON LABS.,INC. (72-3701)				112,000
6X1	CLINICAL TRIALS - PHASE III	- 20% -	22,400	
C	TRANSFUSION RESEARCH	20%	22,400	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 80% -	89,600	
L	LEUKEMIA	80%	89,600	
HAZLETON LABS.,INC. (74-3702)				311,235
6X2	TOXICOLOGY/PHARMACOLOGY	- 100% -		
A	ANIMAL STUDIES	100%	311,235	
HAZLETON RES.ANIM., INC. (73-879)				1,272
3X1	PHARMACOLOGY/TOXICOLOGY	- 100% -		
A	PROTOCOL TOXICITY STUDIES	50%	636	
B	SPECIAL TOXICITY STUDIES	50%	636	
HEBREW UNIV. (71-2127)				105,000
6X1	CLINICAL TRIALS - PHASE III	- 100% -		
A	BREAST	25%	26,250	
B	COLON	75%	78,750	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
HERNER INFORMATION SERVICES, INC. (74-3720)				$ 162,919
8XX	PROGRAM MANAGEMENT	- 100% -		
B	DISSEMINATION OF INFORMATION	100%	162,919	
HERNER INFORMATION SERVICES, INC. (72-3244)				0
8XX	PROGRAM MANAGEMENT	- 100% -		
B	DISSEMINATION OF INFORMATION	100%		
HORTON LABS. (73-837)				23,400
1XX	ACQUISITION OF MATERIALS	- 14% -	3,276	
E	BIOASSAY OF NATURAL PRODUCTS	2%	468	
F	ANIMALS	12%	2,808	
2AX	BASIC SCREEN	- 63% -	14,742	
C	RELATED NEW ASSAY DEVELOPMENT	3%	702	
D	ANIMALS	60%	14,040	
2B2	VERIFICATION SCREEN	- 23% -	5,382	
E	ANIMALS	23%	5,382	
IIT RESEARCH INST. (74-3755)				116,058
1XX	ACQUISITION OF MATERIALS	- 20% -	23,212	
E	BIOASSAY OF NATURAL PRODUCTS	15%	17,409	
G	DATA PROCESSING AND SUPPORT	5%	5,803	
2AX	BASIC SCREEN	- 80% -	92,846	
A	PRIMARY SCREENING IN VITRO	5%	5,803	
B	PRIMARY SCREENING IN VIVO	55%	63,832	
E	DATA PROCESSING AND SUPPORT	20%	23,212	
IIT RESEARCH INST. (68-1021)				1,000,000
1XX	ACQUISITION OF MATERIALS	- 10% -	100,000	
E	BIOASSAY OF NATURAL PRODUCTS	10%	100,000	
2AX	BASIC SCREEN	- 55% -	550,000	
B	PRIMARY SCREENING IN VIVO	41%	410,000	
C	RELATED NEW ASSAY DEVELOPMENT	4%	40,000	
D	ANIMALS	10%	100,000	
2B2	VERIFICATION SCREEN	- 35% -	350,000	
B	DETAILED DRUG EVALUATION IN VIVO	29%	290,000	
E	ANIMALS	6%	60,000	
IIT RESEARCH INST. (67-1141)				0
3X1	PHARMACOLOGY/TOXICOLOGY	- 100% -		
A	PROTOCOL TOXICITY STUDIES	100%		

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
ILLINOIS, BOARD OF TRUSTEES OF THE UNIVERSITY OF (72-2078)		$	90,000
1XX ACQUISITION OF MATERIALS	- 100% -		
B PLANT PRODUCTS PROCUREMENT	90%	81,000	
C FERMENTATION/ANTIBIOTICS PROCUREMENT	10%	9,000	
ILLINOIS, BOARD OF TRUSTEES OF THE UNIVERSITY OF (72-3208)			68,252
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	50%	34,126	
C FERMENTATION/ANTIBIOTICS PROCUREMENT	50%	34,126	
INDIAN CANCER RES. CENTRE (P.L.480)			0
1XX ACQUISITION OF MATERIALS	- 53% -		
B PLANT PRODUCTS PROCUREMENT	53%		
2AX BASIC SCREEN	- 47% -		
B PRIMARY SCREENING IN VIVO	27%		
D ANIMALS	20%		
INDIANA UNIV.FDN. (70-2028)			29,909
5XX CLINICAL TRIALS - PHASE II	- 30% -	8,973	
J BRAIN	30%	8,973	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	20,936	
J BRAIN	70%	20,936	
INST. OF CANC. RES. ROYAL CANC.HOSPITAL (72-3717)			36,000
2B2 VERIFICATION SCREEN	- 100% -		
A DETAILED DRUG EVALUATION IN VITRO	50%	18,000	
B DETAILED DRUG EVALUATION IN VIVO	50%	18,000	
INST.JULES BORDET (72-2053)			40,000
1XX ACQUISITION OF MATERIALS	- 30% -	12,000	
A SYNTHETICS PROCUREMENT	25%	10,000	
C FERMENTATION/ANTIBIOTICS PROCUREMENT	5%	2,000	
2AX BASIC SCREEN	- 25% -	10,000	
A PRIMARY SCREENING IN VITRO	5%	2,000	
B PRIMARY SCREENING IN VIVO	20%	8,000	
8XX PROGRAM MANAGEMENT	- 45% -	18,000	
B DISSEMINATION OF INFORMATION	45%	18,000	
IST. DI RICHERCHE FARM. "MARIO NEGRI" (72-3242)			58,000
3X1 PHARMACOLOGY/TOXICOLOGY	- 50% -	29,000	
F PHARMACOLOGY	50%	29,000	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
6X2	TOXICOLOGY/PHARMACOLOGY	- 50% -	29,000	
A	ANIMAL STUDIES	50%	29,000	
IST. DI RICHERCHE FARM. 'MARIO NEGRI' (73-3720)			$	149,490
1XX	ACQUISITION OF MATERIALS	- 15% -	22,424	
A	SYNTHETICS PROCUREMENT	15%	22,424	
2AX	BASIC SCREEN	- 85% -	127,066	
B	PRIMARY SCREENING IN VIVO	85%	127,067	
IST. NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (73-3714)				63,500
5XX	CLINICAL TRIALS - PHASE II	- 50% -	31,750	
A	BREAST	25%	15,875	
B	COLON	12%	7,620	
F	STOMACH	13%	8,255	
6X1	CLINICAL TRIALS - PHASE III	- 25% -	15,875	
A	BREAST	20%	12,700	
J	BRAIN	5%	3,175	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 25% -	15,875	
A	BREAST	20%	12,700	
J	BRAIN	5%	3,175	
JACKSON LAB. (74-125)				193,131
1XX	ACQUISITION OF MATERIALS	- 6% -	11,591	
E	BIOASSAY OF NATURAL PRODUCTS	1%	1,931	
F	ANIMALS	5%	9,657	
2AX	BASIC SCREEN	- 40% -	77,251	
C	RELATED NEW ASSAY DEVELOPMENT	3%	5,794	
D	ANIMALS	37%	71,458	
2B2	VERIFICATION SCREEN	- 54% -	104,289	
C	MOLECULAR BIOLOGY PROGRAM	5%	9,657	
D	RELATED NEW ASSAY DEVELOPMENT	49%	94,634	
JAPANESE FD. FOR CANCER RESEARCH (72-2054)				54,660
1XX	ACQUISITION OF MATERIALS	- 10% -	5,466	
A	SYNTHETICS PROCUREMENT	5%	2,733	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	5%	2,733	
2AX	BASIC SCREEN	- 20% -	10,932	
A	PRIMARY SCREENING IN VITRO	2%	1,093	
B	PRIMARY SCREENING IN VIVO	18%	9,839	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
2B2	VERIFICATION SCREEN	- 20% -	10,932	
A	DETAILED DRUG EVALUATION IN VITRO	2%	1,093	
B	DETAILED DRUG EVALUATION IN VIVO	18%	9,839	
4X1	CLINICAL TRIALS - PHASE I	- 30% -	16,398	
B	PHARMACOLOGY/TOXICOLOGY	30%	16,398	
8XX	PROGRAM MANAGEMENT	- 20% -	10,932	
B	DISSEMINATION OF INFORMATION	20%	10,932	
JEFFERSON MED.COLL. (70-2136)				$ 58,000
4X2	CLINICAL PHARMACOLOGY	- 90% -	52,200	
A	CLIN. PHARMACOLOGY & PHARMACOKINETICS	90%	52,200	
6X2	TOXICOLOGY/PHARMACOLOGY	- 10% -	5,800	
A	ANIMAL STUDIES	10%	5,800	
JOHNS HOPKINS UNIV. (74-3718)				43,957
3X1	PHARMACOLOGY/TOXICOLOGY	- 100% -		
E	OTHER	50%	21,979	
F	PHARMACOLOGY	50%	21,979	
KANSAS, UNIV. OF (72-3217)				90,000
2B1	FORMULATION	- 100% -		
A	DEVEL. OF EXPERIMENTAL FORMULATIONS	100%	90,000	
KANSAS, UNIV.OF (71-2010)				88,119
1XX	ACQUISITION OF MATERIALS	- 14% -	12,338	
E	BIOASSAY OF NATURAL PRODUCTS	2%	1,762	
F	ANIMALS	12%	10,574	
2AX	BASIC SCREEN	- 63% -	55,514	
C	RELATED NEW ASSAY DEVELOPMENT	3%	2,644	
D	ANIMALS	60%	52,871	
2B2	VERIFICATION SCREEN	- 23% -	20,267	
D	RELATED NEW ASSAY DEVELOPMENT	3%	2,644	
E	ANIMALS	20%	17,624	
KANSAS,UNIV.OF (71-2184)				100,000
6X2	TOXICOLOGY/PHARMACOLOGY	- 100% -		
B	HUMAN STUDIES	75%	75,000	
C	DATA PROCESSING AND SUPPORT	25%	25,000	
KENTUCKY,UNIV.OF (67-1173)				19,457
5XX	CLINICAL TRIALS - PHASE II	- 30% -	5,838	
J	BRAIN	30%	5,837	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
6X1	CLINICAL TRIALS - PHASE III	- 70% -	13,619	
J	BRAIN	70%	13,620	
LAB. SUPPLY CO. (73-840)				$ 26,550
1XX	ACQUISITION OF MATERIALS	- 14% -	3,718	
E	BIOASSAY OF NATURAL PRODUCTS	2%	531	
F	ANIMALS	12%	3,186	
2AX	BASIC SCREEN	- 63% -	16,726	
C	RELATED NEW ASSAY DEVELOPMENT	3%	797	
D	ANIMALS	60%	15,930	
2B2	VERIFICATION SCREEN	- 23% -	6,106	
E	ANIMALS	23%	6,107	
LAB. SUPPLY CO. (73-835)				10,260
1XX	ACQUISITION OF MATERIALS	- 14% -	1,438	
E	BIOASSAY OF NATURAL PRODUCTS	2%	205	
F	ANIMALS	12%	1,231	
2AX	BASIC SCREEN	- 63% -	6,463	
C	RELATED NEW ASSAY DEVELOPMENT	3%	308	
D	ANIMALS	60%	6,156	
2B2	VERIFICATION SCREEN	- 23% -	2,359	
E	ANIMALS	23%	2,360	
LAB. SUPPLY CO. (70-2041)				133,960
1XX	ACQUISITION OF MATERIALS	- 14% -	18,756	
E	BIOASSAY OF NATURAL PRODUCTS	2%	2,679	
F	ANIMALS	12%	16,075	
2AX	BASIC SCREEN	- 63% -	84,394	
C	RELATED NEW ASSAY DEVELOPMENT	3%	4,019	
D	ANIMALS	60%	80,376	
2B2	VERIFICATION SCREEN	- 23% -	30,810	
D	RELATED NEW ASSAY DEVELOPMENT	3%	4,019	
E	ANIMALS	20%	26,792	
LEO GOODWIN INST. FOR CANCER RESEARCH (73-3729)				184,786
1XX	ACQUISITION OF MATERIALS	- 14% -	25,871	
E	BIOASSAY OF NATURAL PRODUCTS	2%	3,696	
F	ANIMALS	12%	22,174	
2AX	BASIC SCREEN	- 63% -	116,415	
C	RELATED NEW ASSAY DEVELOPMENT	3%	5,544	
D	ANIMALS	60%	110,872	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2B2 VERIFICATION SCREEN	- 23% -	42,500	
E ANIMALS	23%	42,501	
LITTLE,ARTHUR D.INC.(73-3727)			$ 2,144,037
1XX ACQUISITION OF MATERIALS	- 7% -	150,085	
E BIOASSAY OF NATURAL PRODUCTS	7%	150,083	
2AX BASIC SCREEN	- 27% -	578,889	
A PRIMARY SCREENING IN VITRO	1%	21,440	
B PRIMARY SCREENING IN VIVO	23%	493,129	
C RELATED NEW ASSAY DEVELOPMENT	2%	42,881	
D ANIMALS	1%	21,440	
2B2 VERIFICATION SCREEN	- 25% -	536,009	
A DETAILED DRUG EVALUATION IN VITRO	1%	21,440	
B DETAILED DRUG EVALUATION IN VIVO	21%	450,248	
D RELATED NEW ASSAY DEVELOPMENT	2%	42,881	
E ANIMALS	1%	21,440	
3X1 PHARMACOLOGY/TOXICOLOGY	- 41% -	879,054	
C ANIMALS	3%	64,321	
E OTHER	6%	128,642	
F PHARMACOLOGY	32%	686,092	
LITTLE,ARTHUR D.INC.(72-3711)			456,800
1XX ACQUISITION OF MATERIALS	- 10% -	45,680	
H OTHER	10%	45,680	
2B2 VERIFICATION SCREEN	- 20% -	91,360	
C MOLECULAR BIOLOGY PROGRAM	15%	68,520	
D RELATED NEW ASSAY DEVELOPMENT	5%	22,840	
5XX CLINICAL TRIALS - PHASE II	- 20% -	91,360	
S OTHER	20%	91,360	
6X1 CLINICAL TRIALS - PHASE III	- 45% -	205,560	
L LEUKEMIA	10%	45,680	
R PROTECTED ENVIRONMENT	10%	45,680	
T OTHER	25%	114,200	
8XX PROGRAM MANAGEMENT	- 5% -	22,840	
A ADMINISTRATION	5%	22,840	
LITTLE,ARTHUR D.INC.(72-3257)			189,743
1XX ACQUISITION OF MATERIALS	- 20% -	37,949	
G DATA PROCESSING AND SUPPORT	20%	37,949	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2AX	BASIC SCREEN	- 80% -	151,794	
E	DATA PROCESSING AND SUPPORT	80%	151,794	
	LITTON BIONETICS, INC. (72-3294)			$ 431,750
1XX	ACQUISITION OF MATERIALS	- 32% -	138,160	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	31%	133,843	
G	DATA PROCESSING AND SUPPORT	1%	4,318	
2B2	VERIFICATION SCREEN	- 38% -	164,065	
E	ANIMALS	38%	164,065	
8XX	PROGRAM MANAGEMENT	- 30% -	129,525	
A	ADMINISTRATION	30%	129,525	
	LITTON BIONETICS, INC.(73-3741)			319,902
2AX	BASIC SCREEN	- 30% -	95,971	
C	RELATED NEW ASSAY DEVELOPMENT	30%	95,971	
2B2	VERIFICATION SCREEN	- 70% -	223,931	
A	DETAILED DRUG EVALUATION IN VITRO	70%	223,931	
	LITTON BIONETICS, INC.,(72-2062)			145,367
1XX	ACQUISITION OF MATERIALS	- 25% -	36,343	
H	OTHER	25%	36,342	
2B2	VERIFICATION SCREEN	- 25% -	36,341	
C	MOLECULAR BIOLOGY PROGRAM	25%	36,342	
3X1	PHARMACOLOGY/TOXICOLOGY	- 50% -	72,683	
F	PHARMACOLOGY	50%	72,684	
	LITTON BIONETICS, INC.,(71-2340)			2,335,428
1XX	ACQUISITION OF MATERIALS	- 30% -	700,629	
H	OTHER	30%	700,628	
3X1	PHARMACOLOGY/TOXICOLOGY	- 20% -	467,085	
F	PHARMACOLOGY	20%	467,086	
6X2	TOXICOLOGY/PHARMACOLOGY	- 50% -	1,167,714	
B	HUMAN STUDIES	50%	1,167,714	
	MAKERERE UNIV. COLLEGE COUNCIL (67-1343)			178,114
5XX	CLINICAL TRIALS - PHASE II	- 40% -	71,246	
D	PEDIATRIC TUMORS	10%	17,811	
K	LYMPHOMAS	20%	35,623	
N	OTHER DISEASES	10%	17,811	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
6X1 CLINICAL TRIALS - PHASE III	- 60% -	106,868	
D PEDIATRIC TUMORS	20%	35,623	
K LYMPHOMAS	20%	35,623	
N OTHER DISEASES	20%	35,623	
MARSHALL RES.ANIM., INC.(73-889)			$ 0
3X1 PHARMACOLOGY/TOXICOLOGY	- 100% -		
A PROTOCOL TOXICITY STUDIES	50%		
B SPECIAL TOXICITY STUDIES	50%		
MARYLAND,UNIV.OF (74-3748			2,553,000
4X1 CLINICAL TRIALS - PHASE I	- 15% -	382,950	
B PHARMACOLOGY/TOXICOLOGY	15%	382,950	
4X2 CLINICAL PHARMACOLOGY	- 10% -	255,300	
A CLIN. PHARMACOLOGY & PHARMACOKINETICS	10%	255,300	
5XX CLINICAL TRIALS - PHASE II	- 15% -	382,950	
G TESTICULAR	2%	51,060	
J BRAIN	3%	76,590	
K LYMPHOMAS	4%	102,120	
L LEUKEMIA	3%	76,590	
N OTHER DISEASES	3%	76,590	
6X1 CLINICAL TRIALS - PHASE III	- 15% -	382,950	
G TESTICULAR	2%	51,060	
J BRAIN	2%	51,060	
K LYMPHOMAS	4%	102,120	
L LEUKEMIA	4%	102,120	
N OTHER DISEASES	3%	76,590	
6X2 TOXICOLOGY/PHARMACOLOGY	- 10% -	255,300	
A ANIMAL STUDIES	5%	127,650	
B HUMAN STUDIES	5%	127,650	
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 30% -	765,900	
G TESTICULAR	3%	76,590	
J BRAIN	4%	102,120	
K LYMPHOMAS	8%	204,240	
L LEUKEMIA	8%	204,240	
N OTHER DISEASES	7%	178,710	
8XX PROGRAM MANAGEMENT	- 5% -	127,650	
A ADMINISTRATION	5%	127,650	
MASON RES. INST. (71-2259)			630,755
1XX ACQUISITION OF MATERIALS	- 2% -	12,617	
E BIOASSAY OF NATURAL PRODUCTS	2%	12,615	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2AX BASIC SCREEN	- 63% -	397,375	
B PRIMARY SCREENING IN VIVO	60%	378,453	
C RELATED NEW ASSAY DEVELOPMENT	3%	18,923	
2B2 VERIFICATION SCREEN	- 35% -	220,763	
B DETAILED DRUG EVALUATION IN VIVO	30%	189,227	
F DATA PROCESSING AND SUPPORT	5%	31,538	
MASON RES.INST. (70-2055)			$ 151,693
3X1 PHARMACOLOGY/TOXICOLOGY	- 100% -		
A PROTOCOL TOXICITY STUDIES	70%	106,185	
B SPECIAL TOXICITY STUDIES	30%	45,508	
MAYO FOUNDATION (71-2185)			127,000
5XX CLINICAL TRIALS - PHASE II	- 100% -		
A BREAST	100%	127,000	
MAYO FOUNDATION (70-2066)			88,000
5XX CLINICAL TRIALS - PHASE II	- 100% -		
B COLON	90%	79,200	
E PANCREAS	5%	4,400	
F STOMACH	5%	4,400	
MAYO FOUNDATION (73-3735)			126,465
5XX CLINICAL TRIALS - PHASE II	- 20% -	25,294	
C LUNG	20%	25,293	
6X1 CLINICAL TRIALS - PHASE III	- 30% -	37,939	
C LUNG	30%	37,940	
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 50% -	63,232	
C LUNG	50%	63,233	
MEMORIAL HOSPITAL FOR CANCER & ALLIED DISEASES (72-2073)			0
5XX CLINICAL TRIALS - PHASE II	- 50% -		
A BREAST	50%		
6X1 CLINICAL TRIALS - PHASE III	- 50% -		
A BREAST	50%		
MERCK & COMPANY (72-2002)			516,688
2B4 PROC. OF PRECLINICAL MATERIALS	- 75% -	387,516	
A SYNTHETICS PROCUREMENT	75%	387,516	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 25% -	129,172	
A	SYNTHETICS PRODUCTION	25%	129,172	
MIAMI,	UNIV. OF (70-2225)			$ 154,100
1XX	ACQUISITION OF MATERIALS	- 2% -	3,082	
E	BIOASSAY OF NATURAL PRODUCTS	2%	3,082	
2AX	BASIC SCREEN	- 98% -	151,018	
A	PRIMARY SCREENING IN VITRO	98%	151,018	
MICHIGAN,	REGENTS OF THE UNIV. OF (69-2004)			25,000
1XX	ACQUISITION OF MATERIALS	- 100% -		
A	SYNTHETICS PROCUREMENT	100%	25,000	
MICHIGAN,	REGENTS OF THE UNIVERSITY OF (72-3714)			0
3X1	PHARMACOLOGY/TOXICOLOGY	- 100% -		
F	PHARMACOLOGY	100%		
MICHIGAN,	STATE OF, DEPT. OF PUBLIC HEALTH (73-3702)			47,746
2AX	BASIC SCREEN	- 100% -		
A	PRIMARY SCREENING IN VITRO	100%	47,746	
MICRO.	ASSOC. INC. (69-2177)			70,000
6X2	TOXICOLOGY/PHARMACOLOGY	- 100% -		
D	OTHER	100%	70,000	
MICRO.	ASSOC.,INC. (73-3728)			1,527,098
1XX	ACQUISITION OF MATERIALS	- 26% -	397,050	
A	SYNTHETICS PROCUREMENT	14%	213,794	
G	DATA PROCESSING AND SUPPORT	12%	183,252	
2AX	BASIC SCREEN	- 15% -	229,064	
B	PRIMARY SCREENING IN VIVO	13%	198,523	
D	ANIMALS	2%	30,542	
2B2	VERIFICATION SCREEN	- 44% -	671,921	
B	DETAILED DRUG EVALUATION IN VIVO	25%	381,775	
D	RELATED NEW ASSAY DEVELOPMENT	2%	30,542	
E	ANIMALS	3%	45,813	
G	OTHER	14%	213,794	
3X1	PHARMACOLOGY/TOXICOLOGY	- 15% -	229,063	
C	ANIMALS	4%	61,084	
F	PHARMACOLOGY	11%	167,981	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
MIDWEST RES. INST. (73-3743)				$ 344,915
1XX	ACQUISITION OF MATERIALS	- 94% -	324,221	
A	SYNTHETICS PROCUREMENT	94%	324,220	
2AX	BASIC SCREEN	- 6% -	20,694	
A	PRIMARY SCREENING IN VITRO	6%	20,695	
MIDWEST RES. INST. (73-3722)				214,381
2B4	PROC. OF PRECLINICAL MATERIALS	- 20% -	42,877	
F	OTHER	20%	42,876	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 80% -	171,504	
G	ANALYTICAL AND QUALITY CONTROL	80%	171,505	
MILES LABORATORIES (74-3709)				0
8XX	PROGRAM MANAGEMENT	- 100% -		
B	DISSEMINATION OF INFORMATION	100%		
MISSOURI,CURATORS OF THE UNIVERSITY OF(71-2323)				138,434
5XX	CLINICAL TRIALS - PHASE II	- 100% -		
Q	MARKERS	75%	103,826	
R	DATA PROCESSING AND SUPPORT	25%	34,609	
MONSANTO RES.CORP. (73-3709)				267,237
2B4	PROC. OF PRECLINICAL MATERIALS	- 10% -	26,724	
B	PLANT PRODUCTS PROCUREMENT	10%	26,724	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 90% -	240,513	
B	PLANT PRODUCTS PRODUCTION	90%	240,513	
MONSANTO RES.CORP. (72-3715)				210,000
2B4	PROC. OF PRECLINICAL MATERIALS	- 80% -	168,000	
E	RADIOLABELED MATERIALS PROCUREMENT	80%	168,000	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 20% -	42,000	
J	OTHER	20%	42,000	
MOUNT SINAI SCHOOL OF MEDICINE (74-3703)				49,878
5XX	CLINICAL TRIALS - PHASE II	- 50% -	24,939	
M	OVARY	50%	24,939	
6X1	CLINICAL TRIALS - PHASE III	- 50% -	24,939	
M	OVARY	50%	24,939	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
MURPHY BREEDING LABS. (73-868)				$ 8,672
1XX	ACQUISITION OF MATERIALS	- 14% -	1,215	
E	BIOASSAY OF NATURAL PRODUCTS	2%	173	
F	ANIMALS	12%	1,041	
2AX	BASIC SCREEN	- 63% -	5,463	
C	RELATED NEW ASSAY DEVELOPMENT	3%	260	
D	ANIMALS	60%	5,203	
2B2	VERIFICATION SCREEN	- 23% -	1,994	
E	ANIMALS	23%	1,995	
N.Y. STATE DEPT OF HEALTH (73-3734)				165,118
6X1	CLINICAL TRIALS - PHASE III	- 40% -	66,048	
C	LUNG	40%	66,047	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 60% -	99,070	
C	LUNG	60%	99,071	
N.Y. STATE DEPT. OF HEALTH(73-3726)				97,500
2AX	BASIC SCREEN	- 35% -	34,125	
A	PRIMARY SCREENING IN VITRO	10%	9,750	
B	PRIMARY SCREENING IN VIVO	25%	24,375	
3X1	PHARMACOLOGY/TOXICOLOGY	- 65% -	63,375	
F	PHARMACOLOGY	65%	63,375	
N.Y. STATE DEPT.OF HEALTH (72-2072)				37,800
5XX	CLINICAL TRIALS - PHASE II	- 50% -	18,900	
A	BREAST	50%	18,900	
6X1	CLINICAL TRIALS - PHASE III	- 50% -	18,900	
A	BREAST	50%	18,900	
N.Y. STATE DEPT.OF HEALTH (70-2035)				97,128
1XX	ACQUISITION OF MATERIALS	- 14% -	13,599	
F	ANIMALS	14%	13,598	
2AX	BASIC SCREEN	- 63% -	61,190	
D	ANIMALS	57%	55,363	
F	OTHER	6%	5,828	
2B2	VERIFICATION SCREEN	- 23% -	22,339	
E	ANIMALS	20%	19,426	
G	OTHER	3%	2,914	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
NATIONAL ACADEMY OF SCIENCES #16(64-44)			$ 0
8XX PROGRAM MANAGEMENT	- 100% -		
B DISSEMINATION OF INFORMATION	100%		
NATIONAL ACADEMY OF SCIENCES #6(64-44)			17,500
8XX PROGRAM MANAGEMENT	- 100% -		
B DISSEMINATION OF INFORMATION	100%	17,500	
NEW JERSEY COLLEGE OF MED.& DENTISTRY (72-3211)			87,500
1XX ACQUISITION OF MATERIALS	- 14% -	12,250	
E BIOASSAY OF NATURAL PRODUCTS	2%	1,750	
F ANIMALS	12%	10,500	
2AX BASIC SCREEN	- 63% -	55,125	
C RELATED NEW ASSAY DEVELOPMENT	3%	2,625	
D ANIMALS	60%	52,500	
2B2 VERIFICATION SCREEN	- 23% -	20,125	
D RELATED NEW ASSAY DEVELOPMENT	3%	2,625	
E ANIMALS	20%	17,500	
NEW YORK UNIV. (67-1174)			32,965
5XX CLINICAL TRIALS - PHASE II	- 30% -	9,890	
J BRAIN	30%	9,890	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	23,075	
J BRAIN	70%	23,076	
NOTRE DAME,UNIV.OF (72-3718)			110,000
2B2 VERIFICATION SCREEN	- 100% -		
G OTHER	100%	110,000	
OHIO STATE UNIV. (67-1175)			24,241
5XX CLINICAL TRIALS - PHASE II	- 30% -	7,273	
J BRAIN	30%	7,272	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	16,968	
J BRAIN	70%	16,969	
OHIO STATE UNIV. (73-3736)			142,000
6X1 CLINICAL TRIALS - PHASE III	- 40% -	56,800	
C LUNG	40%	56,800	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 60% -	85,200	
C	LUNG	60%	85,200	
	OREGON STATE UNIV. (72-3201)			$ 73,271
6X2	TOXICOLOGY/PHARMACOLOGY	- 100% -		
A	ANIMAL STUDIES	100%	73,271	
	PARKE, DAVIS & CO. (74-3754)			527,130
1XX	ACQUISITION OF MATERIALS	- 85% -	448,061	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	83%	437,518	
G	DATA PROCESSING AND SUPPORT	2%	10,543	
2AX	BASIC SCREEN	- 15% -	79,069	
A	PRIMARY SCREENING IN VITRO	10%	52,713	
C	RELATED NEW ASSAY DEVELOPMENT	5%	26,357	
	PARKE, DAVIS & CO. (72-3708)			267,000
2B4	PROC. OF PRECLINICAL MATERIALS	- 50% -	133,500	
A	SYNTHETICS PROCUREMENT	50%	133,500	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 50% -	133,500	
A	SYNTHETICS PRODUCTION	25%	66,750	
B	PLANT PRODUCTS PRODUCTION	25%	66,750	
	PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (71-2138)			13,383
2AX	BASIC SCREEN	- 30% -	4,015	
C	RELATED NEW ASSAY DEVELOPMENT	30%	4,015	
2B2	VERIFICATION SCREEN	- 70% -	9,368	
C	MOLECULAR BIOLOGY PROGRAM	70%	9,368	
	PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (73-3719)			521,310
1XX	ACQUISITION OF MATERIALS	- 100% -		
G	DATA PROCESSING AND SUPPORT	100%	521,310	
	PENNWALT CORP. (71-2309)			30,000
1XX	ACQUISITION OF MATERIALS	- 100% -		
A	SYNTHETICS PROCUREMENT	100%	30,000	
	PFIZER, CHAS. & CO. INC. (73-3234)			156,210
1XX	ACQUISITION OF MATERIALS	- 100% -		
H	OTHER	100%	156,210	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
PHARM-ECO (72-3707)			$ 160,000
2B4 PROC. OF PRECLINICAL MATERIALS	- 40% -	64,000	
A SYNTHETICS PROCUREMENT	40%	64,000	
3X2 DRUG PROD. & FORM. FOR CLIN. TRIALS	- 60% -	96,000	
A SYNTHETICS PRODUCTION	60%	96,000	
PHILIPS ROXANE LABS.(71-2139)			64,959
2B1 FORMULATION	- 20% -	12,992	
A DEVEL. OF EXPERIMENTAL FORMULATIONS	20%	12,992	
3X2 DRUG PROD. & FORM. FOR CLIN. TRIALS	- 80% -	51,967	
F FORMULATION	60%	38,975	
G ANALYTICAL AND QUALITY CONTROL	20%	12,992	
POMONA COLLEGE (73-3703)			39,198
1XX ACQUISITION OF MATERIALS	- 100% -		
H OTHER	100%	39,198	
RESEARCH FD. OF STATE UNIV. OF NEW YORK (73-3715)			227,900
5XX CLINICAL TRIALS - PHASE II	- 10% -	22,790	
R DATA PROCESSING AND SUPPORT	10%	22,790	
6X1 CLINICAL TRIALS - PHASE III	- 25% -	56,975	
S DATA PROCESSING AND SUPPORT	25%	56,975	
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 35% -	79,765	
P DATA PROCESSING AND SUPPORT	35%	79,765	
8XX PROGRAM MANAGEMENT	- 30% -	68,370	
A ADMINISTRATION	20%	45,580	
B DISSEMINATION OF INFORMATION	10%	22,790	
RESEARCH TRIANGLE INST.(69-2019)			137,000
1XX ACQUISITION OF MATERIALS	- 95% -	130,150	
B PLANT PRODUCTS PROCUREMENT	75%	102,750	
C FERMENTATION/ANTIBIOTICS PROCUREMENT	15%	20,550	
E BIOASSAY OF NATURAL PRODUCTS	5%	6,850	
2AX BASIC SCREEN	- 5% -	6,850	
A PRIMARY SCREENING IN VITRO	5%	6,850	
RHODE ISLAND, UNIV. OF (71-2312)			0
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%		

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
SCRIPPS CLINIC & RES. FD. (72-2042)				$ 49,131
1XX	ACQUISITION OF MATERIALS	- 75% -	36,849	
A	SYNTHETICS PROCUREMENT	75%	36,848	
2AX	BASIC SCREEN	- 25% -	12,282	
A	PRIMARY SCREENING IN VITRO	13%	6,387	
B	PRIMARY SCREENING IN VIVO	12%	5,896	
SHEEHAN INST. FOR RES. (72-3719)				25,000
1XX	ACQUISITION OF MATERIALS	- 100% -		
A	SYNTHETICS PROCUREMENT	100%	25,000	
SIMONSEN LABS. (72-3219)				178,738
1XX	ACQUISITION OF MATERIALS	- 14% -	25,025	
E	BIOASSAY OF NATURAL PRODUCTS	2%	3,575	
F	ANIMALS	12%	21,449	
2AX	BASIC SCREEN	- 63% -	112,604	
C	RELATED NEW ASSAY DEVELOPMENT	3%	5,362	
D	ANIMALS	60%	107,243	
2B2	VERIFICATION SCREEN	- 23% -	41,109	
E	ANIMALS	23%	41,110	
SIMONSEN LABS. (73-816)				24,300
1XX	ACQUISITION OF MATERIALS	- 14% -	3,402	
E	BIOASSAY OF NATURAL PRODUCTS	2%	486	
F	ANIMALS	12%	2,916	
2AX	BASIC SCREEN	- 63% -	15,309	
C	RELATED NEW ASSAY DEVELOPMENT	3%	729	
D	ANIMALS	60%	14,580	
2B2	VERIFICATION SCREEN	- 23% -	5,589	
D	RELATED NEW ASSAY DEVELOPMENT	3%	729	
E	ANIMALS	20%	4,860	
SIMONSEN LABS. (71-2101)				109,789
1XX	ACQUISITION OF MATERIALS	- 14% -	15,371	
E	BIOASSAY OF NATURAL PRODUCTS	2%	2,196	
F	ANIMALS	12%	13,175	
2AX	BASIC SCREEN	- 63% -	69,167	
C	RELATED NEW ASSAY DEVELOPMENT	3%	3,294	
D	ANIMALS	60%	65,873	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2B2	VERIFICATION SCREEN	- 23% -	25,251	
E	ANIMALS	23%	25,251	
	SMALL BUSINESS ADMINISTRATION (74-3719)		$	77,872
1XX	ACQUISITION OF MATERIALS	- 10% -	7,788	
H	OTHER	10%	7,787	
2B2	VERIFICATION SCREEN	- 90% -	70,084	
C	MOLECULAR BIOLOGY PROGRAM	30%	23,362	
D	RELATED NEW ASSAY DEVELOPMENT	60%	46,723	
	SOUTH SHORE ANALYT. & RES.LAB.(64-932)			0
3X1	PHARMACOLOGY/TOXICOLOGY	- 100% -		
A	PROTOCOL TOXICITY STUDIES	90%		
B	SPECIAL TOXICITY STUDIES	10%		
	SOUTHERN ANIM.FARMS (73-363)			24,750
1XX	ACQUISITION OF MATERIALS	- 14% -	3,466	
E	BIOASSAY OF NATURAL PRODUCTS	2%	495	
F	ANIMALS	12%	2,970	
2AX	BASIC SCREEN	- 63% -	15,592	
C	RELATED NEW ASSAY DEVELOPMENT	3%	743	
D	ANIMALS	60%	14,850	
2B2	VERIFICATION SCREEN	- 23% -	5,692	
E	ANIMALS	23%	5,693	
	SOUTHERN ANIM.FARMS (72-2013)			110,000
1XX	ACQUISITION OF MATERIALS	- 7% -	7,700	
F	ANIMALS	7%	7,700	
2AX	BASIC SCREEN	- 45% -	49,500	
C	RELATED NEW ASSAY DEVELOPMENT	5%	5,500	
D	ANIMALS	40%	44,000	
2B2	VERIFICATION SCREEN	- 48% -	52,800	
D	RELATED NEW ASSAY DEVELOPMENT	5%	5,500	
E	ANIMALS	43%	47,300	
	SOUTHERN CALIF. UNIVERSITY OF (72-3241)			128,100
6X2	TOXICOLOGY/PHARMACOLOGY	- 100% -		
A	ANIMAL STUDIES	75%	96,075	
C	DATA PROCESSING AND SUPPORT	25%	32,025	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
SOUTHERN RES.INST. (73-3712)			$ 327,250
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	327,250	
SOUTHERN RES.INST. (66-29)			82,500
2B2 VERIFICATION SCREEN	- 100% -		
C MOLECULAR BIOLOGY PROGRAM	100%	82,500	
SOUTHERN RES.INST. (65-654)			94,712
3X1 PHARMACOLOGY/TOXICOLOGY	- 85% -	80,506	
D DATA PROCESSING AND SUPPORT	10%	9,471	
F PHARMACOLOGY	75%	71,034	
6X2 TOXICOLOGY/PHARMACOLOGY	- 15% -	14,206	
A ANIMAL STUDIES	15%	14,207	
SOUTHERN RES.INST. (70-2083)			338,333
3X1 PHARMACOLOGY/TOXICOLOGY	- 40% -	135,334	
B SPECIAL TOXICITY STUDIES	40%	135,333	
6X2 TOXICOLOGY/PHARMACOLOGY	- 60% -	202,999	
A ANIMAL STUDIES	60%	203,000	
SOUTHERN RES.INST. (71-2098)			126,149
2AX BASIC SCREEN	- 30% -	37,846	
A PRIMARY SCREENING IN VITRO	6%	7,569	
B PRIMARY SCREENING IN VIVO	16%	20,184	
C RELATED NEW ASSAY DEVELOPMENT	8%	10,092	
2B2 VERIFICATION SCREEN	- 70% -	88,303	
A DETAILED DRUG EVALUATION IN VITRO	3%	3,784	
B DETAILED DRUG EVALUATION IN VIVO	40%	50,460	
D RELATED NEW ASSAY DEVELOPMENT	27%	34,060	
SPARTAN RES. ANIM. INC.(73-878)			0
1XX ACQUISITION OF MATERIALS	- 14% -		
E BIOASSAY OF NATURAL PRODUCTS	2%		
F ANIMALS	12%		
2AX BASIC SCREEN	- 63% -		
C RELATED NEW ASSAY DEVELOPMENT	3%		
D ANIMALS	60%		

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
2B2	VERIFICATION SCREEN	- 23% -		
E	ANIMALS	23%		
SPARTAN RES. ANIM., INC. (73-858)				$ 0
1XX	ACQUISITION OF MATERIALS	- 14% -		
E	BIOASSAY OF NATURAL PRODUCTS	2%		
F	ANIMALS	12%		
2AX	BASIC SCREEN	- 63% -		
C	RELATED NEW ASSAY DEVELOPMENT	3%		
D	ANIMALS	60%		
2B2	VERIFICATION SCREEN	- 23% -		
D	RELATED NEW ASSAY DEVELOPMENT	3%		
E	ANIMALS	20%		
STANFORD RES. INST. (73-3742)				432,994
1XX	ACQUISITION OF MATERIALS	- 96% -	415,675	
A	SYNTHETICS PROCUREMENT	96%	415,674	
2AX	BASIC SCREEN	- 4% -	17,319	
A	PRIMARY SCREENING IN VITRO	4%	17,320	
STANFORD RES.INST. (73-3723)				342,436
2B4	PROC. OF PRECLINICAL MATERIALS	- 20% -	68,488	
F	OTHER	20%	68,487	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 80% -	273,948	
G	ANALYTICAL AND QUALITY CONTROL	80%	273,949	
STANFORD RES.INST. (72-3200)				73,010
6X2	TOXICOLOGY/PHARMACOLOGY	- 100% -		
A	ANIMAL STUDIES	100%	73,010	
STANFORD RES.INST. (71-2162)				200,000
2B4	PROC. OF PRECLINICAL MATERIALS	- 80% -	160,000	
E	RADIOLABELED MATERIALS PROCUREMENT	80%	160,000	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 20% -	40,000	
J	OTHER	20%	40,000	
STARKS ASSOC., INC. (72-3203)				339,062
2B4	PROC. OF PRECLINICAL MATERIALS	- 60% -	203,438	
A	SYNTHETICS PROCUREMENT	60%	203,437	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 40% -	135,624	
A	SYNTHETICS PRODUCTION	40%	135,625	
STARKS,C.P.,INC. (72-3705)				$ 174,665
1XX	ACQUISITION OF MATERIALS	- 100% -		
A	SYNTHETICS PROCUREMENT	100%	174,665	
TACONIC FARMS (73-912)				18,375
1XX	ACQUISITION OF MATERIALS	- 14% -	2,573	
E	BIOASSAY OF NATURAL PRODUCTS	2%	368	
F	ANIMALS	12%	2,205	
2AX	BASIC SCREEN	- 63% -	11,576	
C	RELATED NEW ASSAY DEVELOPMENT	3%	551	
D	ANIMALS	60%	11,025	
2B2	VERIFICATION SCREEN	- 23% -	4,226	
E	ANIMALS	23%	4,226	
TECHNICON INSTRUMENTS CORP. (73-3746)				0
5XX	CLINICAL TRIALS - PHASE II	- 100% -		
Q	MARKERS	100%		
TENNESSEE,UNIV.OF (70-2044)				75,000
2B1	FORMULATION	- 25% -	18,750	
A	DEVEL. OF EXPERIMENTAL FORMULATIONS	25%	18,750	
3X2	DRUG PROD. & FORM FOR CLIN. TRIALS	- 75% -	56,250	
F	FORMULATION	65%	48,750	
G	ANALYTICAL AND QUALITY CONTROL	10%	7,500	
TEXAS INBRED MICE (68-88)				32,428
1XX	ACQUISITION OF MATERIALS	- 14% -	4,541	
E	BIOASSAY OF NATURAL PRODUCTS	6%	1,946	
F	ANIMALS	8%	2,594	
2AX	BASIC SCREEN	- 63% -	20,429	
C	RELATED NEW ASSAY DEVELOPMENT	5%	1,621	
D	ANIMALS	58%	18,808	
2B2	VERIFICATION SCREEN	- 23% -	7,458	
D	RELATED NEW ASSAY DEVELOPMENT	3%	973	
E	ANIMALS	20%	6,486	
TEXAS INBRED MICE (74-3711)				503,372
1XX	ACQUISITION OF MATERIALS	- 14% -	70,473	
E	BIOASSAY OF NATURAL PRODUCTS	2%	10,067	
F	ANIMALS	12%	60,405	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2AX	BASIC SCREEN	- 63% -	317,124	
C	RELATED NEW ASSAY DEVELOPMENT	3%	15,101	
D	ANIMALS	60%	302,023	
2B2	VERIFICATION SCREEN	- 23% -	115,775	
E	ANIMALS	23%	115,776	
	TEXAS, UNIV. OF (73-3737)			$ 0
5XX	CLINICAL TRIALS - PHASE II	- 20% -		
C	LUNG	20%		
6X1	CLINICAL TRIALS - PHASE III	- 40% -		
C	LUNG	40%		
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 40% -		
C	LUNG	40%		
	TEXAS, UNIV. OF (73-3710)			190,000
5XX	CLINICAL TRIALS - PHASE II	- 70% -	133,000	
M	OVARY	70%	133,000	
6X1	CLINICAL TRIALS - PHASE III	- 30% -	57,000	
M	OVARY	30%	57,000	
	TEXAS,UNIV.OF (66-1156)			493,993
3X1	PHARMACOLOGY/TOXICOLOGY	- 50% -	246,998	
F	PHARMACOLOGY	50%	246,997	
4X2	CLINICAL PHARMACOLOGY	- 10% -	49,399	
A	CLIN. PHARMACOLOGY & PHARMACOKINETICS	10%	49,399	
6X2	TOXICOLOGY/PHARMACOLOGY	- 40% -	197,596	
A	ANIMAL STUDIES	10%	49,399	
B	HUMAN STUDIES	30%	148,198	
	TEXAS,UNIV.OF (71-2094)			253,814
6X1	CLINICAL TRIALS - PHASE III	- 100% -		
R	PROTECTED ENVIRONMENT	100%	253,814	
	TRACOR JITCO, INC. (73-3744)			0
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 100% -		
H	DATA PROCESSING AND SUPPORT	100%		
	TRW, INC. (71-2190)			30,000
3X1	PHARMACOLOGY/TOXICOLOGY	- 100% -		
A	PROTOCOL TOXICITY STUDIES	50%	15,000	
B	SPECIAL TOXICITY STUDIES	50%	15,000	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
TULANE UNIVERSITY (73-3701)				$ 129,990
2B2	VERIFICATION SCREEN	- 95% -	123,491	
D	RELATED NEW ASSAY DEVELOPMENT	5%	6,500	
E	ANIMALS	90%	116,991	
3X1	PHARMACOLOGY/TOXICOLOGY	- 5% -	6,499	
C	ANIMALS	5%	6,500	
UNIV. HOSPITAL (73-3725)				0
5XX	CLINICAL TRIALS - PHASE II	- 100% -		
E	PANCREAS	50%		
Q	MARKERS	50%		
UPJOHN COMPANY (73-3707)				93,822
1XX	ACQUISITION OF MATERIALS	- 48% -	45,037	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	45%	42,220	
E	BIOASSAY OF NATURAL PRODUCTS	3%	2,815	
2AX	BASIC SCREEN	- 25% -	23,454	
A	PRIMARY SCREENING IN VITRO	10%	9,382	
B	PRIMARY SCREENING IN VIVO	7%	6,568	
C	RELATED NEW ASSAY DEVELOPMENT	7%	6,568	
E	DATA PROCESSING AND SUPPORT	1%	938	
2B2	VERIFICATION SCREEN	- 10% -	9,382	
C	MOLECULAR BIOLOGY PROGRAM	10%	9,382	
4X2	CLINICAL PHARMACOLOGY	- 17% -	15,949	
B	DATA PROCESSING AND SUPPORT	17%	15,950	
UPJOHN COMPANY (72-3709)				6,000
1XX	ACQUISITION OF MATERIALS	- 100% -		
A	SYNTHETICS PROCUREMENT	100%	6,000	
UPJOHN COMPANY (74-3753)				346,275
1XX	ACQUISITION OF MATERIALS	- 57% -	197,379	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	55%	190,451	
G	DATA PROCESSING AND SUPPORT	2%	6,926	
2AX	BASIC SCREEN	- 17% -	58,866	
A	PRIMARY SCREENING IN VITRO	7%	24,239	
B	PRIMARY SCREENING IN VIVO	3%	10,388	
C	RELATED NEW ASSAY DEVELOPMENT	7%	24,239	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2B1	FORMULATION	- 1% -	3,462	
A	DEVEL. OF EXPERIMENTAL FORMULATIONS	1%	3,463	
2B2	VERIFICATION SCREEN	- 10% -	34,627	
C	MOLECULAR BIOLOGY PROGRAM	10%	34,628	
4X2	CLINICAL PHARMACOLOGY	- 15% -	51,941	
A	CLIN. PHARMACOLOGY & PHARMACOKINETICS	13%	45,016	
B	DATA PROCESSING AND SUPPORT	2%	6,926	
UTAH, UNIV. OF (72-3710)				$ 40,000
1XX	ACQUISITION OF MATERIALS	- 82% -	32,800	
A	SYNTHETICS PROCUREMENT	82%	32,800	
2AX	BASIC SCREEN	- 18% -	7,200	
A	PRIMARY SCREENING IN VITRO	18%	7,200	
VALUE ENGINEERING COMPANY (73-3706)				696,386
1XX	ACQUISITION OF MATERIALS	- 14% -	97,495	
G	DATA PROCESSING AND SUPPORT	14%	97,494	
2AX	BASIC SCREEN	- 60% -	417,831	
E	DATA PROCESSING AND SUPPORT	60%	417,832	
2B2	VERIFICATION SCREEN	- 26% -	181,060	
F	DATA PROCESSING AND SUPPORT	26%	181,060	
VERMONT, UNIV. OF (73-37[illegible]6)				26,927
5XX	CLINICAL TRIALS - PHASE II	- 50% -	13,465	
R	DATA PROCESSING AND SUPPORT	50%	13,464	
6X1	CLINICAL TRIALS - PHASE III	- 50% -	13,462	
S	DATA PROCESSING AND SUPPORT	50%	13,464	
VETERANS ADMIN. (64-4C)				1,264,000
5XX	CLINICAL TRIALS - PHASE II	- 10% -	126,400	
C	LUNG	10%	126,400	
6X1	CLINICAL TRIALS - PHASE III	- 30% -	379,200	
C	LUNG	30%	379,200	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 50% -	632,000	
B	COLON	21%	265,440	
C	LUNG	21%	265,440	
E	PANCREAS	6%	75,840	
N	OTHER DISEASES	2%	25,280	
8XX	PROGRAM MANAGEMENT	- 10% -	126,400	
A	ADMINISTRATION	2%	25,280	
B	DISSEMINATION OF INFORMATION	8%	101,120	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
VIRGINIA, UNIV.OF (71-2099)				$ 209,000
1XX	ACQUISITION OF MATERIALS	- 100% -		
B	PLANT PRODUCTS PROCUREMENT	100%	209,000	
WALTER REED ARMY MED.CENTER(64-5)				51,524
4X2	CLINICAL PHARMACOLOGY	- 2% -	1,032	
A	CLIN. PHARMACOLOGY & PHARMACOKINETICS	2%	1,030	
5XX	CLINICAL TRIALS - PHASE II	- 46% -	23,701	
A	BREAST	40%	20,610	
C	LUNG	1%	515	
H	PROSTATE	2%	1,030	
J	BRAIN	2%	1,030	
R	DATA PROCESSING AND SUPPORT	1%	515	
6X1	CLINICAL TRIALS - PHASE III	- 29% -	14,941	
A	BREAST	20%	10,305	
G	TESTICULAR	8%	4,122	
S	DATA PROCESSING AND SUPPORT	1%	515	
6X2	TOXICOLOGY/PHARMACOLOGY	- 2% -	1,030	
B	HUMAN STUDIES	2%	1,030	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 21% -	10,820	
A	BREAST	20%	10,305	
P	DATA PROCESSING AND SUPPORT	1%	515	
WARF INST., INC. (67-0747)				7,490
1XX	ACQUISITION OF MATERIALS	- 100% -		
B	PLANT PRODUCTS PROCUREMENT	100%	7,490	
WARF INST., INC. (66-494)				563,867
1XX	ACQUISITION OF MATERIALS	- 39% -	219,909	
E	BIOASSAY OF NATURAL PRODUCTS	39%	219,908	
2AX	BASIC SCREEN	- 56% -	315,765	
B	PRIMARY SCREENING IN VIVO	56%	315,766	
2B2	VERIFICATION SCREEN	- 5% -	28,193	
B	DETAILED DRUG EVALUATION IN VIVO	5%	28,193	
WARF INST., INC. (74-3707)				81,196
1XX	ACQUISITION OF MATERIALS	- 100% -		
B	PLANT PRODUCTS PROCUREMENT	100%	81,196	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
WASH.STATE UNIV. (72-2059)		$	0
1XX ACQUISITION OF MATERIALS	- 14% -		
E BIOASSAY OF NATURAL PRODUCTS	4%		
F ANIMALS	10%		
2AX BASIC SCREEN	- 63% -		
C RELATED NEW ASSAY DEVELOPMENT	3%		
D ANIMALS	60%		
2B2 VERIFICATION SCREEN	- 23% -		
D RELATED NEW ASSAY DEVELOPMENT	3%		
E ANIMALS	20%		
WASHINGTON HOSPITAL CENTER			0
6X2 TOXICOLOGY/PHARMACOLOGY	- 100% -		
A ANIMAL STUDIES	100%	26,885	
WASHINGTON,UNIV.OF (71-2193)			0
6X2 TOXICOLOGY/PHARMACOLOGY	- 100% -		
A ANIMAL STUDIES	100%		
WISCONSIN,BOARD OF REGENTS OF THE UNIVERSITY OF (73-3[illegible]33)			38,000
1XX ACQUISITION OF MATERIALS	- 100% -		
E BIOASSAY OF NATURAL PRODUCTS	100%	38,000	
WISCONSIN,UNIV.OF (71-2275)			123,705
1XX ACQUISITION OF MATERIALS	- 50% -	61,853	
A SYNTHETICS PROCUREMENT	50%	61,853	
3X1 PHARMACOLOGY/TOXICOLOGY	- 50% -	61,852	
F PHARMACOLOGY	50%	61,853	
YALE UNIVERSITY (71-2339)			76,000
1XX ACQUISITION OF MATERIALS	- 75% -	57,000	
A SYNTHETICS PROCUREMENT	75%	57,000	
2AX BASIC SCREEN	- 25% -	19,000	
A PRIMARY SCREENING IN VITRO	12%	9,120	
B PRIMARY SCREENING IN VIVO	13%	9,880	
YALE UNIVERSITY (73-3740)			0
3X1 PHARMACOLOGY/TOXICOLOGY	- 100% -		
A PROTOCOL TOXICITY STUDIES	90%		
B SPECIAL TOXICITY STUDIES	10%		

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
YALE UNIVERSITY (73-3711)				$ 23,920
2AX	BASIC SCREEN	- 67% -	16,027	
A	PRIMARY SCREENING IN VITRO	67%	16,026	
4X1	CLINICAL TRIALS - PHASE I	- 16% -	3,827	
B	PHARMACOLOGY/TOXICOLOGY	16%	3,827	
4X2	CLINICAL PHARMACOLOGY	- 17% -	4,066	
A	CLIN. PHARMACOLOGY & PHARMACOKINETICS	17%	4,066	

TABLE IV
DESCRIPTION OF CONTRACTS IN THE DIVISION OF CANCER TREATMENT PROGRAM

ABBOTT LABORATORIES (N01-CM-43704)

This contract was designed to develop a conditioning medium that will cause normal and leukemic granulocytic precursor cells to differentiate and form colonies of mature granulocytes. The Contractor will supply large amounts of active purified conditioning factor that will be considered acceptable for clinical trials. The material will have potential clinical use in leukemia, aplastic anemia and drug-induced aplasias.

ABCOR, INC. (N01-CM-02239)

This contract provides engineering investigations and development to significantly improve the yields of human blood cells, particularly leukocytes, from hematologically normal donors, (1) through modifications of the NCI-IBM Blood Cell Separator or its operational techniques, or (2) through entirely new systems of procurement based upon different principles of blood cell separation such as centrifugal elutriation, filtration leukapheresis, etc.

AEROJET-GENERAL CORPORATION (N01-CM-81300)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

AGRICULTURE, U. S. DEPARTMENT OF (TRANSFER OF FUNDS - Y01-CM-40001)

Under the Transfer of Funds agreement with the Medicinal Plant Resoures Laboratory of the U.S.D.A., collects approximately 4,000 new plant materials per year for screening. Plant samples are obtained from all parts of the world.

The plants collected by the U.S.D.A. are shipped to the extraction contractor, WARF Institute, Inc. As confirmed active plants appear, the U.S.D.A. recollects in large quantity, on a priority based on the needs of the Division of Cancer Treatment Program, at the rate of up to 200 recollections per year for shipment to the fractionating chemists. Very large recollections are made, as requested by Drug Research and Development, for the preparation of clinical quantities of drugs. Also, up to 200 large collections are made of species, not previously screened, that are members of families known to be rich sources of activity.

AGRICULTURE, U. S. DEPARTMENT OF (TRANSFER OF FUNDS - Y01-CM-40001) (CONTIN.)

The nomenclature of all plants acquisitioned is being reviewed and corrected. A computer file of all plants is being completed and, with this and with our screening and isolation data, a full review of the plant program as a basis for improving sample collections is being conducted.

ALBANY MEDICAL COLLEGE (N01-CM-22074)

This contract is designed to evaluate the effects of chemotherapy on the growth and size of breast cancer lesions, toxicity and survival. A study comparing CCNU and 5-Azacytidine has been completed with 2/21 regressions, and 2/19 with CCNU. Thirty-two patients have been entered by Albany comparing Adriamycin with adrenalectomy or combination of Cytoxan, 5-Fluorouracil and Prednisone (CFP). An additional 46 patients have been entered on this study by Roswell Park. Among the presently evaluable patients, there are responses in 8/22 with Adriamycin, 11/25 with CFP, and 6/18 with adrenalectomy.

ALDRICH CHEMICAL COMPANY (N01-CM-23706)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. About 30% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

ALLEGHENY GENERAL HOSPITAL (N01-CM-43730)

The objective of this contract is to determine the dose and time course effects of antitumor drugs selected by the NCI, on survival of normal gastrointestinal crypts and on the *in vivo* survival of experimental murine mastocytoma cells. The effects of multiple drug dose interval on the normal gastrointestinal mucosa and the mastocytoma (in various metastatic sites) will be evaluated. Information on dose schedules and drug combinations leading to minimal or maximal gastrointestinal toxicity will be provided concurrent with the effects of these therapies on a proliferating murine mastocytoma. The methods used in these studies to determine gastrointestinal crypt survival were developed by the Contractor.

ARIZONA BOARD OF REGENTS (ARIZONA STATE UNIVERSITY) (N01-CM-12308)

The major objective of this contract is the preparation, from insects, marine animal and plant specimens, of extracts suitable for bioassay in the NCI antitumor screen. The raw natural product specimens are obtained in most instances by the Contractor with emphasis on animal and marine sources. The extracts are prepared in accordance with procedures acceptable to NCI and in amounts suitable for routine *in vivo* screening. Extracts showing preliminary activity are prepared anew for confirmatory testing and materials showing confirmed activity are assigned for fractionation and isolation.

ARIZONA BOARD OF REGENTS (UNIVERSITY OF ARIZONA) (NO1-CM-33750)

The major objective of this contract is the fractionation of confirmed active plants in order to isolate in a pure state and identify the antitumor active components. Recent promising results have been obtained from the extraction of plants collected in Greece, Hawaii and Arizona. Further fractionation is being carried out to isolate the active components. A KB cell culture unit has been added to expedite the fractionation work.

ARIZONA, UNIVERSITY OF (NO1-CM-33713)

The second year of this contract (begun 11/10/73) was devoted to the further development of a staging system for patients with multiple myeloma. The rationale for this proposed staging system was based on detailed studies by the Principal Investigator, Dr. Sydney Salmon, in working out a procedure for determining total myeloma cell number in patients. The proposed system incorporates available clinical and laboratory data and measurements in conjunction with tumor cell number to derive a means for staging myeloma patients. This system is now being tested prospectively to determine if the desired correlations with actual tumor cell measurements hold true. It is anticipated this system could be used for evaluation of the number of tumor cells remaining following therapy using clinical parameters without the necessity for carrying out the detailed and involved procedures for actual measurement of this number.

ARS/SPRAGUE-DAWLEY (NO1-CM-02044)

This rodent production center contract supports a production effort designed to furnish animals in accordance with laboratory requirements. Breeding animals are furnished by the Government. Major emphasis is directed toward AKR mouse production.

ARS/SPRAGUE-DAWLEY (NO1-CM-12007)

This primary genetic center produces a variety of outbred, inbred and hybrids of inbred rodents. All production activities are effected in closely controlled environments. The overall workscope comprises four distinct tasks identified as follows:

Task 1 provides inbred, outbred and hybrid rodents in modified conventional environments.

Task 2 provides Syrian golden hamsters with defined but non-pathogenic organisms. A small percentage of the effort is devoted to the development of germ-free hamsters and gerbils.

Task 3 provides inbred and outbred laboratory strains of rats as germ-free and/or with defined microflora.

Task 4 provides various germ-free inbred, outbred and hybrids of inbred rodents, and the production of counterparts of the various species with defined microflora.

ARS/SPRAGUE-DAWLEY (NO1-CM-12342)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. Major emphasis is upon the production of AKR mice.

ASH STEVENS, INC. (NO1-CM-12314)

The objective of this contract is the synthesis of potential antitumor oxygen and/or sulfur heterocyclic compounds. The relatively unexplored areas of 1,2,4-trithiolanes cyclic disulfides and trisulfides are being examined as carriers for sulfur and oxygen heterocyclics, metabolically active functions and antimetabolites. Advantage will be taken of any leads which develop during the course of the work to extend the synthesis into new potentially active structural areas.

ASSOCIATED BIOMEDIC SYSTEMS (NO1-CM-02271)

The purposes of this contract are: (1) to supply normal human blood lymphocytes, both untreated and stimulated with phytohemagglutinin; (2) to produce and evaluate human myeloid colony-stimulating factor(s) by "conditioning" media with various cells and then testing such media for "factor" activity; (3) to perform various services regarding culture of human cell lines such as (a) mass production of "interesting" lines (e.g., cells containing RNA-directed DNA polymerase or cell producing "factors"), and (b) ridding cell lines of contaminating mycoplasma by various techniques; (4) procurement of sera and cells from pat tumors, and of hematopoietic tissue (e.g., bone marrow and spleen) from normal individuals. About 250 billion lymphocytes were provided last year. Certain cell-free "conditioned" media have been identified as rich sources of colony-stimulating factor. At least one cell line has been freed of detectable mycoplasma recently.

ATOMIC ENERGY COMMISSION (Y01-CM-10056)

The principal objective of this project is to study the cell cycle phase specificity of clinical drugs and drugs in development toward clinical trials. Drugs are evaluated for their ability to selectively inhibit progress through G_1, S, G_2, and M- phases of their proliferative cycle and for their selective cytocidal effects in these phases, as well. Both synchronized and exponentially growing mammalian cell culture systems are used. The importance of such studies stems from the need to identify and develop drugs which may be specifically cytotoxic to non-proliferating but viable and clonogenic cells. The *in vitro* methodologies used were developed by the Contractor.

ATOMIC ENERGY COMMISSION (Y01-CM-10059)

This is an interagency agreement for support of a joint effort of the AEC Cooperative Group to study localization and to determine the usefulness of Gallium-67 in Hodgkin's disease and other lymphomas, as well as lung cancer. Immediate objective is designed to determine the value of this agent as an additional diagnostic aid for staging and determining the effectiveness and completeness of treatment. A new Project Officer has been named in the absence of Dr. R. M. Knisely. Agreement has been amended to continue through June 30, 1974.

ATOMIC ENERGY COMMISSION (Y01-CM-10060)

This contract involves the study of potential biologic markers for cancer in body fluids by means of high resolution high-pressure ion exchange chromatography. Initial efforts have focused on minor and methylated nucleic acid derivatives (tRNA catabolic end products) in urine. Sensitive analytical tools utilized in addition to high-pressure chromatography include gas chromatography, gas chromatography-mass spectrometry, and high- and low-resolution mass spectrometry. Preliminary quantitative results indicated elevated levels for specific nucleosides in the urine of approximately 60% of the cancer patients studied. Further efforts are focused on the analysis of specific serum protein bound sugars (utilizing high-pressure liquid chromatographic separation) in the blood of patients with malignancies. In addition, studies are in progress to determine the compounds represented by abnormal peaks obtained on chromatograms from patients with various selected types of cancer.

ATOMIC ENERGY COMMISSION (Y01-CM-40101)

The objective of this effort is to develop a blood cell separation method which will collect sufficient granulocytes ($\sim 10^{10}$/day) from normal donors to permit widespread adoption of this technique in granulocyte replacement therapy. A unique design approach which was the end result of an extensive ORNL analysis "Blood Cell Separator Development for In Vivo Leukapheresis" is being followed. This consists of radically modifying the blood flow path of the existing IBM rotor to mechanically enhance rouleax formation and to photoelectrically monitor

ATOMIC ENERGY COMMISSION (YO1-CM-40101) (CONTIN.)

and control separating cell fractions in the interior of the rotor. This feasibility effort covers rotor fabrication and testing with dog models.

ATOMIC ENERGY COMMISSION (YO1-CM-40102)

This agreement will accomplish R&D with instrumental and biological techniques to progressively adapt a prototype cell microfluorometer and computer data analysis system for human tumor cell characterization and drug evaluation studies planned by the NCI. The initial year will accomplish the engineering, fabrication, testing and delivery of a multi-parameter cell microfluorometer and sorting device coupled with a PDP-11/40 computer system as well as interim interface device for data collection and analysis. Also to be conducted in parallel are biological studies concerning "instrument compatible" cell staining, dispersal and fixation techniques on untested cell samples from a variety of human and animal tumors of current NCI interest. During the second year, it is planned to continue the cell preparation work to explore and optimize techniques, and to exploit the instrument's potential for characterizing model animal tumors (L1210 ascites, B16 melanoma, Lewis Lung carcinoma) as well as several human tumors both *in vivo* and *in vitro*.

AUTOMATION INDUSTRIES, INC. (NO1-CM-33717)

This contract provides support for the Medical Oncology area in the collection and computer processing of clinical data. Contractor extracts data from medical records and other clinical sources and assists in the development and maintenance of computer files, and the generation of computer retrievals and reports. During the past year, the Contractor has demonstrated excellent capabilities in the accurate extraction and computerization of medical data.

AUTOMATION INDUSTRIES, INC. (NO1-CM-33732)

This professional services contract was established to provide the necessary administrative and technical support to the Office of the Director, Division of Cancer Treatment. The primary function is the coordination and technical skills involved with the organization of conferences, graphic support and editorial services.

BATTELLE MEMORIAL INSTITUTE (NO1-CM-02008)

The Contractor performs bacteriologic diagnostic services with emphasis upon the detection of *Salmonella spp.* and *Pseudomonas spp.* in program rodents and other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. Approximately 10,000 samples are screened per year. The research portion of this contract (a minor component) is directed towards improvement in cultural techniques and the elucidation of salmonella inhibitory substances associated with certain microorganisms.

BATTELLE MEMORIAL INSTITUTE (NO1-CM-12182)

In vivo testing of new synthetic materials and natural products of both plant and animal origin, as well as the secondary evaluation of active materials of interest to the DR&D program, are conducted at Battelle Memorial Institute. The current rate of effort is at the level of approximately 37,000 tests per annum. The *in vivo* testing is being conducted in accord with the current revised DR&D protocols, i.e., synthetic materials are being injected on days 2 and 6 in mice implanted with the L1210 leukemia and natural products are being injected on days 1-9 in mice implanted with the P388 leukemia. Natural products which have displayed efficacy in the P388 leukemia are also tested in the L1210 leukemia. When requested, Battelle Memorial Institute conducts testing in the B16 Melanoma and the Lewis Lung carcinoma of materials of interest to the DR&D program. This secondary evaluation testing is in addition to schedule dependency studies of materials of interest to the program in which the optimum dose, schedule and route are ascertained. A tumor bank is maintained with the tumors stored available to the DR&D program. Tumor assay systems may be changed by the Project Officer dependent upon the current needs of the DR&D program. At the Project Officer's request, methodology and/or research is conducted.

BATTELLE MEMORIAL INSTITUTE (NO1-CM-12262)

This service-type contract was primarily concerned with the preclinical toxicologic evaluation of two potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies were directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity was characterized as to predictability, reversibility and schedule dependency of the toxicity. These characterizations were based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. All work was carried out in close cooperation with NCI and the results reported to the Laboratory of Toxicology. This contract has been terminated this Fiscal Year.

BATTELLE MEMORIAL INSTITUTE (NO1-CM-43746)

In March 1974, a Prime Contract was awarded with Battelle Memorial Laboratories for supervision of all contract efforts involved in the toxicologic evaluation of potential oncolytic agents under the aegis of the Laboratory of Toxicology. Through the prime contract mechanism, eight (8) contracts formerly carrying out these studies have been consolidated under a single management-type contract. The workscope is comprised of four tasks as follows:

Task I Full Protocol Studies
Task II High Priority Toxicity Studies
Task III Specific Organ Testing
Task IV Automation of Toxicity Data

BATTELLE MEMORIAL INSTITUTE (NO1-CM-92551)

The Contractor maintains a genetic-production center for inbred rodents which provides breeding animals for large-scale laboratory rodent production colonies and limited numbers for testing and research programs. The profile of categories of these animals includes ten strains.

BELLAIRE ACRES (NIH-73-C-805)

This procurement contract is designed to furnish 120,000 six-week-old CD2F$_1$ (BALB/c♀ x DBA/2♂) hybrid mice for Drug Research and Development contract studies. The breeding animals originate from genetic centers.

BELLAIRE ACRES (NIH-73-C-832)

This contract furnishes approximately 404,000 B6D2F$_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

BEN VENUE LABORATORIES, INC. (NO1-CM-43701)

This contract is designed to provide a pharmaceutical facility for the development and production of parenteral clinical dosage forms of antitumor agents. Ben Venue Laboratories, Inc. has the capacity for preparing large-scale production batches of dry-filled, liquid-filled and lyophilized sterile parenteral products. Specifically, the Contractor performs the following duties: (1) formulation development of sterile parenteral products, (2) production and quality assurance testing of parenteral dosage forms for clinical use, (3) stability surveillance of all dosage forms produced, and (4) preparation of manufacture records on all products produced. All projects are initiated by the Project Officer.

BOWMAN GRAY SCHOOL OF MEDICINE (NO1-CM-80950)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

BRISTOL-MYERS COMPANY (NO1-CM-33751)

The major objective of this fermentation contract is the preparation of novel antibiotics. These new antibiotics are obtained using the fermentation

BRISTOL-MEYERS COMPANY (NO1-CM-33751) (CONTIN.)

techniques of biotransformation and co-metabolism of antibiotics, chemicals and plant materials. This effort is to convert materials which have solubility, toxic, or marginal activity problems into worthwhile agents. A multitude of unique organisms are isolated and used to obtain these microbial conversions in an effort to obtain new antineoplastic agents.

BRISTOL-MYERS COMPANY (NO1-CM-90035)

This contract covers a comprehensive fermentation effort to obtain new antitumor agents. The contract includes: (1) preparation of fermentation broths for screening against in vivo systems specified by NCI, (2) a rodent tumor laboratory for in vivo screening and also testing of fractionation and other follow-up studies, (3) the chemical isolation work necessary to obtain purified agents, (4) production of large quantities of agents approved for clinical trials, and (5) the preparation of appropriate dosage forms of such agents. This contract will terminate in May 1974.

BRITISH COLUMBIA, UNIVERSITY OF (NO1-CM-23223)

The major objective of this contract is to develop a general and versatile synthesis of the highly complex and clinically effective dimeric vinca alkaloids, vincristine and vinblastine. The synthesis of some related analogs and derivatives and the preparation of radiolabeled vincristine is being undertaken. In addition, the contract provides for the final isolation, purification and identification of metabolites obtained from samples collected by investigators specified by NCI after in vivo administration of the radiolabeled vincristine.

CALIFORNIA, UNIVERSITY OF (NO1-CM-02090)

This contract was designed to provide HL-A typing analyses on patients and their families as well as typing of selected donors from the Clinical Center Blood Bank and all platelet donors from the Central Blood Service of Baltimore. To date a total of 3,193 typings have been performed. All typings were done and entered into the computerized file on the same day that the blood was received. Thus, HL-A typing results for persons bled in Bethesda were consistently available through the computer terminal by the end of the next day. A total of 590 sera was tested against a panel of 100 standard cells for which HL-A specificities were known, during the past contract year.

CALIFORNIA, UNIVERSITY OF (NO1-CM-43706)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition

CALIFORNIA, UNIVERSITY OF (N01-CM-43706) (CONTIN.)

of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

CAPETOWN, UNIVERSITY OF (N01-CM-33738)

This new contract was designed to conduct studies of intensive multidisciplinary therapy of patients with bronchogenic carcinoma and to determine the efficacy of each of a number of therapeutic approaches, possible relationship to morphologic type and clinical stage of the disease, and the influence of concommitant pathology. It was initiated because of important therapeutic leads which might prolong longevity of patients with lung cancer as the most commonly lethal malignant tumor in the United States as well as many other countries.

CATHOLIC MEDICAL CENTER OF BROOKLYN & QUEENS, INC. (N01-CM-23703)

This contract was initiated to provide an experimental model for human breast cancer therapy. The assay system utilized is the CD_8F_1 spontaneous and first-generation transplanted tumor. During this first contract year drugs have been tested against the first generation transplanted tumor. The drugs being tested are those specified by DCT staff and are those with established clinical activity and candidates for clinical trial. The contract is designed to provide a human tumor model which will enable us to: (1) test drugs to determine those with specificity against breast carcinoma, (2) study the influence of treatment schedule on "slow growing" tumors, and (3) study drug combinations as well as drugs combined with surgery and immunotherapy.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-004-1)

The Central Drug Research Institute, Lucknow, India, collects native Indian medicinal plants, supplies extracts for screening by Drug Research and Development, and fractionates the active extracts. The budget also provides for (1) synthesis of compounds of chemotherapeutic interest, and (2) an antitumor screening laboratory to provide in-house testing capability. Several compounds have been received by DR&D and limited in-house testing has been achieved.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM-02043)

This rodent production center contract supports a production effort designed to furnish animals as required by laboratory programs. Breeding animals are furnished by the Government. Major emphasis is directed toward AKR mouse production.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM-12343)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM-22004)

This primary genetic center has as its objectives the development of germ-free foundation colonies of inbred rodents required for program studies. Pedigreed animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of organisms and are developed as pedigreed expansion colonies in a variety of isolators. Offspring from this second stage are issued to secondary genetic centers which, in turn, produce breeding animals for large-scale prpduction colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring. A large-scale production colony is maintained in order to provide suitable numbers of rodents for laboratory investigators. A colony of agoutis (*Dasyprocta agouti*) is maintained in order to furnish serum for L-asparaginase extraction. Task 1 is concerned with the production of germ-free rodents, counterparts with defined microbial flora and large-scale production; Task 2 in Calco, Italy, produces rodents in bio-containment environments; and Tasks 3 and 4 are concerned with the production of rodents for specific investigations.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-73-C-807)

This procurement contract is designed to furnish 122,000 six-week-old $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice free of pseudomonads for Drug Research and Development contract studies. The breeding animals, which originate from the genetic center at this site, originate from the isolator system and are free from parasites and pathologic organisms.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-73-C-839)

This contract furnishes approximately 409,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

CHEMICAL ABSTRACTS SERVICE (N01-CM-43722)

The Chemical Abstracts Service has participated in the installation of and is operating the first phase of the new Chemical Information System in support of the DR&D accessioning and screening program. This new system provides the improved capability to: (1) identify which compounds are new to the DR&D program; (2) produce computer-generated communications to suppliers, screeners,

CHEMICAL ABSTRACTS SERVICE (NO1-CM-43722) (CONTIN.)

in-house staff, and the acquisition and storage and distribution contractors who are involved in the accessioning and screening process; (3) maintain management, operational and quality control of the accessioning process; and (4) search the files on-line to secure an immediate answer for a possible duplicate sample, analogs of a compound of interest, or a management question. The second phase of the System to be implemented and installed in FY-1975 will automate shipping procedures and inventory control and will automatically identify or assign to specialized testing procedures submissions that duplicate or are closely related to known active compounds.

CHEMICAL ABSTRACTS SERVICE (NO1-CM-51021)

The Chemical Abstracts Service is used for the computer storage and manipulation of chemical information on all compounds screened by NCI for antitumor activities. The structure files now contain approximately 240,000 structural records, with associated names, molecular formula and descriptive information kept in a separate file. All new compounds acquired by the program are checked for novelty against this file, retrospective searches for analogs are performed and name and formula indexes are produced. In addition to this routine work, considerable effort was expended in the past year to prepare for the new chemical system to be operated by CAS under contract NO1-CM-43722. This contract will expire during FY-1974.

CHESTER BEATTY RESEARCH INSTITUTE (NO1-CM-43736)

This is a service contract for the procurement of unique chemicals and drugs from Great Britain and to establish a joint testing program with the Chester Beatty Research Institute. The Contractor will perform all liaison activity required to procure approximately 10,000 samples per year for the NCI screening program. The Contractor will also develop systems involving the growth of human tumors in mice and the evaluation of clinically active drugs against these animal models in order to ascertain levels of correlation. In addition the Contractor will utilize cell culture techniques to study human breast cancer. The level of effort for screening samples will be minimal during the first year with major emphasis being placed in the compound procurement segment of the contract.

CHICAGO, UNIVERSITY OF (NO1-CM-33748)

This contract is intended to support studies to isolate and compare the dihydrofolate reductase from normal and malignant tissues, primarily solid tumors, in order to determine any detectable molecular differences in these enzymes. The study also utilizes _in vitro_ testing to help design inhibitors of human and subhuman dihydrofolate reductase. These are aimed at understanding the mechanism of resistance developed in human malignancy to antifols.

CLEVELAND CLINIC (NO1-CM-33704)

This contract provides support for a protocol study in which patients with severe psoriasis will be randomly allocated to therapy with either methotrexate (MTX) on two different schedules or a control drug. Evaluation of hepatic function will be performed at regular intervals and data will be collected to determine whether hepatotoxicity in psoriasis is due to MTX or is coincidental.

COLLABORATIVE RESEARCH, INC. (NO1-CM-12326)

The objective of this contract is the design and synthesis of oligo and polynucleotides as potential inhibitors of RNA-dependent DNA polymerase and other DNA polymerases. It is anticipated that the synthetic nucleotides may act as templates and primers for different enzymes and may inhibit viral and leukemic DNA polymerases specifically without interfering with the normal nucleic acid metabolism of the host cells.

COLLABORATIVE RESEARCH, INC. (NO1-CM-60506)

The major objective of this contract is the synthesis of some potential metabolites of cyclophosphamide and phosphoramide mustards as latent cytotoxic agents. In addition, the preparation of some analogs and derivatives of homofolic acid is being undertaken. The preparation of homofolic acid in multikilogram quantities was completed.

COLORADO STATE UNIVERSITY (NO1-CM-12192)

The objective of this research-type contract is to investigate the pathogenesis of Adriamycin/Daunomycin-induced cardiotoxicity utilizing clinical, morphological and biochemical parameters. The rabbit appears to be the most promising species for assessing the cardiotoxicity of this class of anticancer agents. Studies have been designed to establish optimal dose and time schedules to provide the highest incidence and maximum consistency of cardiotoxicity. Adriamycin analogs are being screened for cardiotoxicity using the rabbit with the hope of finding one with a maximum therapeutic effect and minimal cardiotoxicity.

COLORADO, UNIVERSITY OF (NO1-CM-12186)

The purposes of this contract are (1) to define the role of transfer RNA methylation in cell differentiation and neoplasis, (2) to determine the nature and role of naturally occurring tRNA methylase inhibitors in these processes, (3) to determine if these inhibitors or synthetic analogs of these natural inhibitors have antineoplastic effects, and (4) to determine if differences in tRNA (or metabolic products of tRNA), in tRNA methylases, or in tRNA methylase inhibitors can provide biochemical markers of neoplasia. Under this contract two natural organ-specific tRNA methylase inhibitor systems have been discovered and the biochemical nature of the inhibitors defined.

CONNECTICUT, UNIVERSITY OF (NO1-CM-12181)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

CONTROL DATA CORPORATION (NO1-CM-33739)

The purpose of this contract is to provide data processing and computer support which enables simple, rapid and on-demand retrieval of clinical information related to the patient data base provided by the participating group of contractors, the Brain Tumor Study Group, who enter patients on study according to specified protocols.

DELAWARE, UNIVERSITY OF (NO1-CM-92115)

This contract has been concerned with biochemical studies on bacterial asparaginases and glutaminases. Principal contract objective for the current year has been to further purify Aerobacter glutaminase and to develop methods for its large-scale production for antitumor and toxicological evaluation. This contract will terminate as of June 30, 1974.

DOW CHEMICAL COMPANY (NO1-CM-12165)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

DOW CHEMICAL COMPANY (NO1-CM-12313)

The objective of this contract is the synthesis of new and novel nitrogen heterocyclic compounds as potential antitumor agents. Specifically, the Contractor is preparing compounds containing the pyrazoloquinazoline, imidazopyrazines, pyrrolopyrazine and pyrazolopyrazine ring systems. These materials are postulated to be antimetabolites. All materials prepared, including intermediates, are submitted for antitumor testing.

DOW CHEMICAL COMPANY (N01-CM-23712)

This service-type contract is concerned primarily with the reproductive studies of three antineoplastic agents in accordance with the FDA Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use, dated January 1966. These studies are divided into three phases: (1) Phase I, general reproductive performance and fertility utilizing mice or rats of both sexes; (2) Phase II, teratology studies using mice and/or rats and rabbits; and (3) Phase III, perinatal studies employing mice and/or rats.

DOW CHEMICAL COMPANY (N01-CM-33724)

It has been demonstrated that some derivatives of rifamycin have significant inhibitory activity for viral and cellular DNA polymerases (including reverse transcriptase). The work of this contract is designed to synthesize rifamycin derivatives which are more effective inhibitors of RNA dependent DNA polymerases. Additionally, the development of a strong selective inhibitor of RNA dependent DNA polymerases could help in clarifying the mechanism of action of this enzyme and possibly lead to new cancer chemotherapy agents through chemical modifications of 3-formylrifamycin-SV.

DUKE UNIVERSITY (N01-CM-02030)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

DUKE UNIVERSITY (N01-CM-22044)

The major objectives of this contract are (1) the development of measures of sensitivity and quantity in radioautography, and (2) the improvement of sensitivity and resolution of the radioautographic process. Because of loss of key personnel at Duke University, completion of data acquisition phase of this contract was delayed. Accordingly, the contract was extended for six months without additional funds in order to permit Duke personnel to complete this phase, employing the radioautographic grain counter developed at NCI. Data analysis has begun on material so developed and will continue so as to be completed on or about January 1, 1975.

FLOW RESEARCH ANIMALS, INC. (NIH-73-C-838)

This contract furnishes approximately 342,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

GEORGE WASHINGTON UNIVERSITY (NO1-CM-12206)

This Contractor is studying the effects of drug combinations in solid spontaneous tumors in dogs. The studies are aimed at determining whether the permeability of methotrexate can be altered with other drugs and whether this drug's biochemical effect can be used to advantage with other drugs that have different chemical properties and modes of action.

GEORGETOWN UNIVERSITY (NO1-CM-12123)

The objective of this program is to conduct virologic studies of patients participating in the National Cancer Institute's chemotherapeutic program to make possible evaluations that will aid the clinician in the selection of measures for supportive care and allow for the development of information on the importance of the role of viruses in the cancer patient in both protected and conventional environments.

GEORGETOWN UNIVERSITY (NO1-CM-33745)

The Eastern Cooperative Oncology Group consists of 350 members and 35 cooperative institutions plus subsidiary hospitals. The Operations Office provides executive and administrative support for activities of the Group. On request from investigators, provides randomization and Group correspondence; obtains data from the investigators and maintains liaison with appropriate members of the NCI staff. The Contractor has continued to function well during the past contract year and has been responsible for changing the activities of the ECOG along more disease-oriented lines.

GEORGETOWN UNIVERSITY (NO1-CM-43732)

This contract has as its main objective the production of novel actinomycins by using biotransformation and co-metabolism fermentation techniques. These novel actinomycins will be produced in pure form in 100-500 mg. quantities and made available for broad tumor screening. The use of many different compounds as substrates will be thoroughly explored.

GEORGIA, UNIVERSITY OF (NO1-CM-12311)

The goal of this contract is the preparation of novel nitrogen heterocyclic compounds for evaluation as potential antitumor agents. The classes currently being emphasized are ethynyl oxazolidones and heteroaromatic azepines. Because of the structural similarities of these materials to those of compounds with known CNS activity, compounds prepared under this contract will be screened in intracerebral as well as intraperitoneal test systems. This contract will terminate in June 1974.

HARLAN INDUSTRIES, INC. (NO1-CM-23218)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. All activities are performed in bio-containment environments.

HARLAN INDUSTRIES, INC. (NIH-73-C-844)

This contract furnishes approximately 122,000 six-week-old CD2F_1 (BALB/c♀ x DBA/2 hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

HARLAN INDUSTRIES, INC. (NIH-73-C-845)

This contract furnishes approximately 409,000 B6D2F_1 (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

HAWAII, UNIVERSITY OF (NO1-CM-33747)

This contract provided originally for the collection of a minimum of 500 plant samples from among the indigenous and exotic higher plants of the Hawaiian Islands and the recollection of larger samples of those that are reproducibly active in the DR&D tests. Plants so obtained are shipped to the extraction contractor, WARF Institute, Inc., and the extracts tested at one of our contract screeners. During the last year, the contract was increased to provide for 1,000 general collections per year, this number to be reduced according to the number of large recollections made.

HAZLETON LABORATORIES, INC. (NO1-CM-23701)

Mixed leukocyte cultures are an important determinant of the degree of compatibility of two individuals relative to transplantation antigens. The first objective of this contract is the selection of MLC and compatible donor recipient pairs for use in an active canine and human marrow grafting and transfusion program; second objective is the continued improvement of the MLC method for use with smaller quantities of patient blood and greater reproducibility; and third objective is the continued immune evaluation of patients on chemotherapy. In addition, a large liquid nitrogen freezer for storage of blood and bone marrow samples will be purchased. This contract has been approved for renewal.

HAZLETON LABORATORIES, INC. (NO1-CM-23704)

This contract provides *in vivo* screening of new test materials, both natural products and synthetic, and the subsequent testing of active materials. The current testing level is at a rate of approximately 50,000 tests per year. The P388 leukemia is routinely used for the initial testing of natural products. Those demonstrating efficacy are also tested in L1210. The L1210 leukemia is routinely used for all new synthetic materials. Limited testing is conducted against the intracerebrally implanted ependymoblastoma. Treatment schedule studies as well as research and development work is conducted as requested by the Drug Evaluation Branch. The DR&D Protocols are followed for all testing.

HAZLETON LABORATORIES, INC. (NO1-CM-33705)

This contract provides for microbiological services in support of the NCI, Division of Cancer Treatment, clinical investigations at the Baltimore Cancer Research Center, and for special testing pertinent to this program. The services have also included microbiological surveillance and environmental studies in support of the Laminar Air Flow Room protocols of the National Cancer Institute, NIH, Bethesda, Maryland. Preliminary results of the studies are teletyped to the BCRC and NCI daily and used as indicated in research microbiological management of patients. The program employs a dataphone system for presumptive reporting and computer techniques for the retrieval of information as required by clinicians and research investigators, as well as final summarized microbiological reports for patients' medical records.

HAZLETON LABORATORIES, INC. (NO1-CM-33708)

The major objectives of this contract are: (1) to obtain model tumor systems in primates in order to ascertain the potential usefulness of various anticancer agents in man; (2) to develop biological markers and diagnostic tests for detecting preneoplastic changes as well as frank neoplasia; (3) to obtain comparative data on the response of primates to known rodent carcinogens and to materials suspected to be carcinogenic in man; (4) to evaluate the long-term effects of antineoplastic agents which are being used clinically for long-term remissions and in the treatment of diffuse collagen disorders; (5) to make available normal and tumor-bearing animals for pharmacologic, toxicologic, biochemical and immunological studies; and (6) to maintain a breeding colony of various species of primates so that these primates may be readily available for use. All work is carried out in close cooperation with the Laboratory of Chemical Pharmacology, NCI.

HAZLETON LABORATORIES, INC. (NO1-CM-43702)

This contract provides for the supply of dogs for leukocyte transfusion and transplantation experiments. Contractor supplies animals with spontaneous neoplasms in order to evaluate the immunotherapeutic effectiveness of bone marrow transplantation and immunotherapeutic manipulation following chemotherapy regimens.

HAZLETON RESEARCH ANIMALS, INC. (NIH-73-C-879)

This contract provides 360 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

HEBREW UNIVERSITY (HADASSAH MEDICAL SCHOOL) (N01-CM-12127)

This research contract is concerned with the effects of an extract (MER - methanol extractable residue) obtained from attenuated tubercle bacilli (BCG) in stimulating the immune mechanism in animals and man with particular emphasis on antitumor activity. During the past year, clinical studies on 17 patients with AML on chemotherapy were randomized to receive, or not to receive, MER. Clinical results are promising. Immune evaluation is underway.

HERNER INFORMATION SERVICES, INC. (N01-CM-23244)

This contract provides for the preparation, printing and distribution of *Cancer Chemotherapy Abstracts* (Volumes 13 and 14, for 1972 and 1973). The price of the annual subscriptions pays the cost of photocomposition, printing and mailing by the Contractor. This current awareness service, published monthly, supplies abstracts and annotations of the world's literature pertinent to cancer chemotherapy.

HERNER INFORMATION SERVICES, INC. (N01-CM-43720)

The purpose of this contract is to prepare Volume 15 of *Cancer Chemotherapy Abstracts* which abstracts the entire literature (both foreign and domestic) of chemotherapy.

HORTON LABORATORIES, INC. (NIH-73-C-837)

This contract furnishes approximately 244,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

IIT RESEARCH INSTITUTE (N01-CM-43755)

This represents a support services contract to provide assistance to the staff in preliminary data evaluation and follow-up on materials found active in the presumptive screen. The Contractor participates with staff in monitoring testing and in expediting work on those materials which appear to have the greatest promise, based upon initial activity.

IIT RESEARCH INSTITUTE (NO1-CM-71141)

This service-type contract was primarily concerned with the preclinical toxicologic evaluation of two/three potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies were directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity was characterized as to predictability, reversibility and schedule dependency of the toxicity. These characterizations were based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity were conducted at the request of the Project Officer. All work was carried out in close cooperation with NCI and the results reported to the Laboratory of Toxicology. This contract has been terminated this Fiscal Year.

IIT RESEARCH INSTITUTE (NO1-CM-81021)

The major portion of this contract provides for the performance of *in vivo* testing of new synthetic materials and natural products, and also for animal health and utilization studies assigned specifically for problems that arise in various screening laboratories and animal supply contractors. Screening is being conducted according to DR&D protocol at a level of approximately 50,000 tests per year. The lymphoid leukemia L1210 in the mouse host is utilized for all synthetics. New natural products are being tested in the P388 leukemia in the mouse host. Testing is conducted in the B16 melanoma tumor systems when requested by DR&D staff. Statistical analysis and characterization studies of the first generation AKR mouse leukemia test system are being completed. Studies in this system are designed to (1) determine the correlation with the spontaneous AKR test system, (2) pick candidates for combination studies, and (3) determine the effectiveness of both early and delayed treatment in this system. Work is beginning with a murine renal carcinoma as a potential organ-targeted system. As a second function, this laboratory also maintains a routine *Salmonella spp.* and *Pseudomonas spp.* diagnostic laboratory and provides general animal disease diagnostic services as requested. These studies include virology, bacteriology, pathology and histopathology. In addition, it includes baseline studies to define the microbiological flora and virus contact status of various species of interest to the program. This portion of the contract provides the quality controls necessary for the maintenance of the quality of the animals utilized in the DR&D program.

Methodology or research and development is carried out only at the request of the Project Officer and during this year has been devoted to the development of new *in vivo* screening tumor test systems and answering specific questions that arose in the *in vivo* screening and animal health and utilization programs.

ILLINOIS, UNIVERSITY OF (NO1-CM-22078)

This contract provides for the fractionation of confirmed active plant extracts and fermentation products in an attempt to isolate in a pure state and identify the active compound(s). Plant materials used in this work are obtained, for the most part, through the U. S. Department of Agriculture.

ILLINOIS, UNIVERSITY OF (NO1-CM-23208)

The principal objectives of this contract are as follows: (1) to provide the Division of Cancer Treatment, NCI, with samples (not previously available) of a number of ansamycin-type antibiotics for biological and related testing. This group includes the streptovaricins, tolypomycins and geldanamycins, at least some of which are highly potent RNA-dependent DNA polymerase inhibitors and also have antiviral activity. Thus, detailed study of as many members of this group as possible is considered imperative; (2) to isolate several presently unidentified streptovaricin components in quantity sufficient for characterization and biological evaluation; and (3) to study the metabolic fate of ansamycin compounds by isolating and identifying products formed after in vivo administration, from samples collected by investigators specified by NCI.

INDIAN CANCER RESEARCH CENTRE (PL-480 - AGREEMENT NO. NIH-01-010-1)

This PL-480 Agreement is for the collection and identification of natural products indigenous to India, the preparation of crude extracts from these materials, and the testing in vivo of these extracts as well as synthetic materials synthesized in India.

For this purpose, an in vivo screening laboratory is maintained in Bombay, India together with an animal breeding colony to supply the necessary host animals. Currently, the L1210 lymphocytic leukemia and the P388 lymphoid leukemia are utilized with testing conducted as per current DR&D protocols. The L1210 tumor system is employed for the testing of synthetic materials with the P388 tumor system employed for the testing of natural products. Those active in this system are tested in the L1210 tumor system.

INDIANA UNIVERSITY FOUNDATION (NO1-CM-02028)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

INSTITUT JULES BORDET (NO1-CM-22053)

The objective of this contract is the maintenance of a liaison office at the Institut Jules Bordet, Cancer Center of the University of Brussels, Belgium. The program is designed to foster close collaboration between European and U. S.

INSTITUT JULES BORDET (NO1-CM-22053) (CONTIN.)

investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy.

INSTITUTE OF CANCER RESEARCH, ROYAL CANCER HOSPITAL (NO1-CM-23717)

The objectives of this contract are to study the effects of chemotherapeutic agents in experimental solid tumor systems in relation to their proliferative state and their vascular supply, and compare the effects of these agents on the hematopoietic system. To date, quantitative in vivo and in vitro clonogenic assays have been developed for B16 melanoma and Lewis Lung carcinoma and for normal marrow. Interrelationships among the effects of nitrosoureas, alkylating agents and radiation have been studied. Studies are in progress comparing cell survival in tumor and marrow following other drugs in single and multiple doses and in two-drug combinations. Radiotracer methods have recently been developed to measure tumor blood supply. These methods will be used to study proliferative and drug response characteristics in tumors in relation to their blood supply.

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION (NO1-CM-12264)

This service-type contract was primarily concerned with the preclinical toxicologic evaluation of one potentially useful antineoplastic agent in accordance with the protocol of the Laboratory of Toxicology. These studies were directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity was characterized as to predictability, reversibility and schedule dependency of the toxicity. These characterizations were based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. All work was carried out in close cooperation with NCI and the results reported to the Laboratory of Toxicology. This contract has been terminated this Fiscal Year.

ISTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI" (NO1-CM-23242)

The purpose of this contract is to develop and refine techniques for the measurement of specific antineoplastic agents and their metabolites in biological tissues and fluids. The overall aim is to determine what concentration of drug or metabolite and time of exposure is responsible for the biochemical disturbance in various tissues which results in retarded growth, reproduction or death. Currently this Contractor is studying adriamycin and its analogues in tumor-bearing mice.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (NO1-CM-33714)

The purpose of this contract is the evaluation of new anticancer drugs alone or in combination with conventional agents in prospective controlled clinical trials. Four studies are being performed: adjuvant combination chemotherapy for breast cancer with positive axillary nodes; controlled study with multiple drug combinations for metastatic breast cancer; controlled study of new chemotherapeutic regimens in advanced carcinoma of stomach, colon and rectum; and a controlled study of BCNU, CCNU and irradiation in the treatment of malignant glioma.

JACKSON LABORATORY (NIH-74-C-125) (FORMERLY NIH-72-C-1171)

This service contract provides for the procurement of approximately 67,000 C57BL/6 and 10,400 DBA/2 inbred mice to screening contractors for tumor transplantation and as breeders for large-scale hybrid production colonies. It also supplies 78,000 AKR inbred mice for compound evaluation studies. Breeding animals are supplied by the Contractor.

JAPANESE FOUNDATION FOR CANCER RESEARCH (NO1-CM-22054)

The objective of this contract is the maintenance of a liaison office at the Japanese Foundation for Cancer Research in Tokyo. The program is designed to foster close collaboration between Japanese and U. S. investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy.

JEFFERSON MEDICAL COLLEGE (NO1-CM-02136)

The purpose of this contract is to obtain information on the disposition and metabolism of new agents entering Phase I clinical trial. A number of agents, including dibromodulcitol, 5-azacytidine, streptozotocin, and currently Yoshi 864, have been studied under this contract. Work is currently proceeding on the latter compound, using radiolabeled material to determine its metabolism and disposition. This contract will be re-competed in FY-1975.

JOHNS HOPKINS UNIVERSITY (NO1-CM-43718)

The synthetic and catabolic rates of specific myeloma paraprotein will be studied utilizing radioactive guanidoarginine for labeling purposes in mice bearing a transplanted myeloma cell line. Efforts will be made to correlate total cell number with the above parameters in an attempt to work out a possible method for determining this number. Comparison will be made to an existing system which requires culture of the involved cells to determine synthetic rate.

KANSAS, UNIVERSITY OF (N01-CM-12010)

This Contractor maintains a genetic production center. Its most important function is to secure the maintenance of the strains of rodents that are of interest in Drug Research and Development program investigations. Its spectrum includes six strains: C57BL/6, DBA/2, AKR, BALB/c, C3H/He and A/He. Animals not required for perpetuation of pedigree are supplied to screening contractors and as breeders for production colonies. Hybrids for special studies are produced as required for specific studies.

KANSAS, UNIVERSITY OF (N01-CM-12184)

The principal objective of this contract is to collect pharmacokinetic data on new and established antitumor agents in patients undergoing treatment for malignant disease and to analyze these data for individual variability which can be correlated with clinical response or some other pharmacologic parameter. Methotrexate, 5-fluorouracil and melphalan are currently underway.

KANSAS, UNIVERSITY OF (N01-CM-23217)

This contract provides research capabilities in the area of formulation design and development. The research involves the development of intravenous dosage forms of new drug substances having formulation problems. A second objective of this research contract is to develop new and unique delivery systems for poorly manageable drugs. The drugs to be researched are assigned by NCI.

KENTUCKY, UNIVERSITY OF (N01-CM-71173)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

LABORATORY SUPPLY COMPANY, INC. (N01-CM-02041)

This Contractor maintains a genetic production colony of six inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished to large-scale production colonies.

LABORATORY SUPPLY COMPANY, INC. (NIH-73-C-835)

This contract furnishes approximately 122,000 six-week-old $CD2F_1$ (BALB/c♀ x DBA/2 hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

LABORATORY SUPPLY COMPANY, INC. (NIH-73-C-840)

This contract furnishes approximately 357,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (NO1-CM-33729)

This primary genetic center has as its objectives the development of germ-free foundation colonies of inbred rodents. Pedigree animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of non-pathogenic organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to secondary genetic centers, which in turn, produce breeding animals for large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small-scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies.

LITTLE, ARTHUR D., INC. (NO1-CM-23257)

This project encompasses the review and analysis of the present Drug Research and Development Biological Data Processing System. The objectives are to provide recommendations for improving and expanding the present system in order to better evaluate and summarize primary screening data for the selection of compounds for secondary screening, to develop methods for the evaluation and reporting of compounds tested in the secondary screen, and for incorporating the secondary screening data into the present system. A primary goal is to provide suppliers with reports in a form of most use to them; this year a brief English language statement indicating whether the compound showed activity in our present screen according to current criteria was added to the Screening Data Summary. This contract expires May 31, 1974.

LITTLE, ARTHUR D., INC. (NO1-CM-23711)

This contract provides broad biomedical engineering resources which are responsive to the support of individual research projects within the Division of Cancer Treatment. A flexible mechanism of support defines and implements engineering projects in close collaboration with the Division of Cancer Treatment staff in active areas such as: automated microspectometry, cell kinetics modeling, the automation of grain counting and bone marrow colony

LITTLE, ARTHUR D., INC. (NO1-CM-23711) (CONTIN.)

assays, and studies of the protective laminar flow environment for supportive care of leukemic patients. A "core group" of senior engineers continually consults and interacts with the NCI staff on the definition of problems and projects while "project groups" are tailored to implement specific engineering tasks under NCI staff direction.

LITTLE, ARTHUR D., INC. (NO1-CM-33727)

This is a broadly based major research and development program which is directly aligned to the overall mission of the NCI Division of Cancer Treatment program. The task objectives are divided into the following major components.

The Screening and Experimental Therapeutics and Tumor Biology effort provides for routine primary screening in in vivo systems of synthetic and natural products, the detailed evaluation of active drugs, schedule dependence and combination chemotherapy studies and the development of new and improved methods for in vivo screening and detailed drug evaluation. Considerable emphasis is placed on the use of animal models of human tumors for the study of combination chemotherapy and the combination of drugs plus radiation.

The Applied Mathematical and Computer Oriented services provide for the analysis of studies from all appropriate contract laboratories related to the development and finalization of new protocols for screening. The principal objective is to advise Drug Research and Development regarding experimental design, parameters, and criteria for activity and data analysis. The contract segment also conducts studies of correlations between clinical activity and activity in animal tumor assays and advises program with respect to the predictive value of animal systems.

The Cell Culture and Related Methodology work consists of routine in vitro screening, bioassay of natural product fractions, detailed drug evaluation in vitro, and the development of improved assays for natural product bioassay.

The Neuropharmacology studies are aimed at approaching various toxicological and pharmacological problems relating to the treatment of neoplastic diseases of the Central Nervous System. Improved techniques are being sought for the administration of agents used to control neoplastic disease in the CNS.

The Central Nervous System solid tumor studies are concerned with the development and evaluation of transplantable gliomas in the CNS of beagle dogs, together with collaborative studies with clinical and pharmacological groups of the NCI to examine the growth kinetics and the behavior of transplantable gliomas to selected groups.

The Preclinical Toxicology portion of this contract was concerned with the preclinical toxicologic evaluation of three potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies were directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity was characterized as to predictability, reversibility and schedule dependency. These characterizations were based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity were conducted at the request of the Project Officer. All work was carried out in close cooperation with NCI and the results reported to the Laboratory of Toxicology. This segment of the total A. D. Little contract has been terminated during this Fiscal Year.

The Physiologic Disposition and Biochemistry effort provides for sophisticated exploratory research on the mode of action and physiological disposition of active agents, and includes a cooperative project with selected clinical groups to measure the metabolic fate and physiologic disposition of drugs being employed in the management of cancer in humans.

The Cryobiology and Tumor Distribution activities provide for the maintenance of a frozen tumor and cell bank, distribution of standard tumor lines to screening laboratories and other research institutions, and methodological studies on the frozen, stored tumor lines.

The Electron Microscopy and Radioautography program provides such services in support of the pharmacological, microbiological and solid tumor studies.

LITTON BIONETICS, INC. (NO1-CM-12340)

The objectives of this project are to develop new approaches for chemotherapy of acute leukemia by searching for specific inhibitors of RNA dependent DNA polymerase and of other leukemic (human) DNA polymerases to expand basic research efforts in tumor cell biochemistry in general in order to achieve a better understanding of the neoplastic process and its prevention and treatment. Techniques are being developed to detect these polymerases and related proteins and nucleic acids *in situ* in cells in an effort to improve diagnosis and prognostication in human leukemia.

LITTON BIONETICS, INC. (NO1-CM-22062)

The purposes of this contract are (1) to provide gram quantities of normal and transformed embryonic tissue culture cells for biochemical studies related to the biochemical mechanisms involved in neoplasia, (2) to provide gram quantities of fresh human leukocytes, normal and leukemic, for biochemical analysis after short-term tissue culture, (3) to evaluate possible biological effects of biochemically identified RNA tumor virus-related components from human leukemic cells, (4) to carry out special metabolic studies of cultured cells in support of biochemical studies, including treatment of cultured cells with various compounds and radioisotope pulse experiments, and (5) to carry out special procedures for the preparation of RNA tumor viruses. Biological materials provided under this contract have contributed directly or indirectly to many biochemical studies accomplished by the Laboratory of Tumor Cell Biology.

LITTON BIONETICS, INC. (NO1-CM-23294)

As one project at the Frederick Cancer Research Center, the Division of Cancer Treatment is developing a fermentation pilot plant for the large-scale production and isolation of microbial products of interest to the program. Facilities will also be available for the production of other natural products.

LITTON BIONETICS, INC. (NO1-CM-33741)

This contract provides a source for the screening of selected drugs for inhibitory activity for selected mammalian cell and viral nucleotide polymerase enzymes (*in vitro*) and for toxicity or specific inhibition of oncogenic viral replication and transforming capacity in cell culture systems. Enzymes employed are the Simian Sarcoma virus reverse transcriptase, mammalian DNA polymerases I and II, RNA polymerases I and II and Poly-A polymerase. Cell culture effects on DNA, RNA and protein synthesis, cell proliferation and murine sarcoma virus focus formation in 3T3 cells are measured. The screening operation includes a preliminary cell culture and anti-enzymatic screen for all compounds and a secondary cell culture and anti-enzymatic evaluation for compounds demonstrating significant activity in the primary screen. Materials submitted to these screens are selected by the Project Officer.

MAKERERE UNIVERSITY COLLEGE (NO1-CM-71343)

This contract was developed to operate the Lymphoma Treatment Center and the Solid Tumor Center, collectively known as the Uganda Cancer Institute, to provide facilities for direct collaborative studies to the members of the NCI, including Medical Oncology, Laboratory of Pharmacology, Immunology, Pathology and Etiology. Research is continuing as previously but under the direction of Dr. Sebastian Kyalwazi and Dr. Charles Olweny.

"MARIO NEGRI" INSTITUTE FOR PHARMACOLOGICAL RESEARCH (NO1-CM-33720)

The main purpose of this project is to establish a regional European contract as an extension of the DCT compound acquisition and screening program. The Contractor will collect approximately 1,500 materials in Southern European countries and perform primary screening at a rate of approximately 7,500 L1210-equivalency tests per year.

MARSHALL RESEARCH ANIMALS, INC. (NIH-73-C-889)

This contract provides 360 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

MARYLAND, UNIVERSITY OF (NO1-CM-43748)

Under this service contract, the University of Maryland at Baltimore will provide suitable facilities, administrative support, ancillary support services and personnel for the inpatient operation of the Baltimore Cancer Research Center, as well as space for activities such as outpatient department, cell component therapy, patient care pharmacy and laboratory efforts necessary for the conduct of the program.

MASON RESEARCH INSTITUTE (NO1-CM-02055)

This service-type contract was primarily concerned with the preclinical toxicologic evaluation of nine potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies were directed toward defining the qualitative and quantitative toxocity of new drugs. The toxicity was characterized as to predictability, reversibility and schedule dependency. These characterizations were based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity were conducted at the request of the Project Officer. All work was carried out in close cooperation with NCI and the results reported to the Laboratory of Toxicology. This contract has been terminated this Fiscal Year.

MASON RESEARCH INSTITUTE (NO1-CM-12259)

This service contract is for the *in vivo* testing of synthetic and natural products which are submitted to DR&D. The L1210 tumor system is utilized for the testing of synthetic materials with natural products tested initially in the P388 leukemia with those demonstrating efficacy being tested in the L1210 leukemia. Testing is proceeding at a level of approximately 37,000 L1210 equivalent tests per year. Schedule dependency studies to determine the optimal dose, schedule and route of administration as well as combination studies are conducted as directed by DR&D staff.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CM-43712)

The objectives of this project are (1) the unique conceptual division of the bleomycin molecule into five complex but synthetically obtainable intermediates, (2) the synthesis of each of the unique parts for biological testing and comparison with the analogous part from the natural product where possible, (3) the determination of the correct stereochemistry for those six centers still unknown, and (4) the joining of the five parts, one at a time in the proper sequence, in order to determine whether all five parts are necessary for antitumor activity. The Contractor will submit samples of intermediates as well as final products for *in vivo* and *in vitro* evaluation by the NCI. These compounds will be fully characterized as to purity and structure.

MAYO FOUNDATION (NO1-CM-02066)

The purpose of this contract is the investigation of new drugs in the treatment of advanced gastrointestinal tumors as a cooperative venture between the Mayo Foundation and the National Cancer Institute. The objective is to have each drug evaluated in a minimum of 20 cases of adenocarcinoma of the colon to meet the requirements of the Division of Cancer Treatment Program linear array. Provision has been made for reporting of data on each trial in a manner which is consistent with the data retrieval currently employed intramurally at NCI. This will enable both direct comparisons and possible integration of data on new drugs from both of these sources.

MAYO FOUNDATION (NO1-CM-12185)

This contract was designed to provide information on patients on the effect of chemotherapy in the growth and size of breast cancer lesions and toxicity. Selection of drugs and protocol will be mutually agreeable to the Project Officer and the Mayo Foundation. Progress to date has been excellent. In sequential studies the response rates have been as follows: (1) Study A - TIC mustard 2/20 vs. CCNU 2/21; (2) Study B - Methyl-CCNU 1/22 vs. Cytoxan, Fluorcuracil and Prednisone (CFP) 7/11 vs. CFP + Vincristine (CFPV) 5/10; (3) Study C - Ifosfamide 4/20 vs. CFP 7/11 vs. CFVP 3/9; (4) Study D - Adriamycin 9/20 vs. CFP 7/16 vs. CFPV 5/12; (5) Study E - Adriamycin + Methotrexate 7/24 vs. CFP 7/12 vs. CFPV 6/10; (6) Study F - Adriamycin, Methotrexate and Vincristine 4/13 vs. CFP 2/5 vs. CFP + Calusterone 1/6. The latter study is ongoing.

MAYO FOUNDATION (NO1-CM-33735)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer and devise and implement therapeutic protocols that will lead to a systemic and progressive understanding of the disease and promote its control. During the past contract year, new drugs and new drug combinations have been explored in patients with distant metastases.

MAYO FOUNDATION (NO1-CM-43715)

This contract is designed to undertake combined modality clinical studies in testicular tumors. The initial protocol treats Stage II nonseminomatous lesions with surgery followed by random allocation to radiotherapy, chemotherapy or radiotherapy plus chemotherapy.

MEMORIAL HOSPITAL (NO1-CM-22073)

This contract was designed to conduct Phase II studies of anticancer agents in patients with metastatic adenocarcinoma of the breast. This was performed as a cooperative venture between Memorial Hospital and the NCI to test selected

MEMORIAL HOSPITAL (N01-CM-22073) (CONTIN.)

agents, either developed by the NCI or of interest to them. A comparison of Adriamycin and CCNU was undertaken with 9/21 responses to Adriamycin and 2/23 responses to CCNU. The contract terminated in February 1974.

MEMORIAL HOSPITAL (N01-CM-43716)

This contract is designed to undertake combined modality clinical studies in testicular tumors. The initial protocol treats Stage II nonseminomatous lesions with surgery followed by random allocation to radiotherapy, chemotherapy or radiotherapy plus chemotherapy.

MERCK AND COMPANY (N01-CM-22002)

This contract provides for the resynthesis of compounds in the amounts needed for clinical trial or for necessary preclinical evaluations. About 25% of the budget is devoted to clinical preparations.

This is the largest and oldest of the Drug Research and Development preparative laboratories. Because of their extensive and diverse experience, the Contractor is able to prepare compounds of many different and difficult types. These are materials for which no commercial source is available or which cannot be prepared in the required quantities by the original supplier.

MIAMI, UNIVERSITY OF (N01-CM-02225)

This contract provides for the *in vitro* presumptive screening of synthetics and natural products and the follow-up tests of natural products having demonstrated correlative activity in the DR&D *in vivo* screening program, as a tool for fractionation. Procedures are those described by DR&D protocols and methodology is carried out only at the request of the Project Officer. Approximately 15,000 tests at three to five dose levels per test are performed yearly.

MICHIGAN DEPARTMENT OF HEALTH (N01-CM-33702)

This contract provides asaay services, utilizing a variety of cell culture methods, as aids in the purification of potential antitumor agents from fermentation broths. The methods employed include paper chromatography and paper electrophoresis in conjunction with cell culture agar diffusion bioautography, as well as growth inhibition assays, both in stationary and suspension cultures. The related fermentation and isolation program serviced by this assay contract is financed by the Michigan Department of Health.

MICHIGAN, REGENTS OF THE UNIVERSITY OF (NO1-CM-23714)

The objective of this contract is to help delineate the role of RNA in DNA replication in human cells. RNA molecules may serve as initiator molecules (primers) in DNA directed (templated) DNA synthesis in phage-bacterial systems and probably in mammalian cells as well. RNA also may direct (template for) DNA synthesis (so called reverse transcriptase reactions); the latter has now been found in RNA tumor viruses, human acute leukemic cells, chick embryo, and in some established tissue culture tumor cell lines. Both types of reactions are potentially relevant to chemotherapy, the former in DNA replication (a target for some useful antitumor agents) and the latter in replication of RNA tumor viruses. Progress in the last year has been made in the identification and preliminary characterization of an RNA primer human lymphoblast cell line. The contract was terminated March 31, 1974.

MICHIGAN, UNIVERSITY OF (NO1-CM-92004)

This contract is for the chemical synthesis of a number of new nitrogen heterocyclic compounds containing complex ring systems. The compounds are for primary screening and are unique structures which have been little explored and/or are heretofore unknown. All materials are fully characterized, of high purity and in sufficient quantity for adequate evaluation. This contract will expire in April 1974.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM-33728)

This contract forms a single coordinated mechanism for the detailed investigation of problems which arise in the chemotherapy of malignant disease. The total program is closely integrated scientifically with NCI operations, and the activities are carried out under the daily guidance of professional staff of Drug Research and Development. Projects selected for study are those which are determined to be highly pertinent to the needs of the preclinical and clinical programs of the NCI. The contract is divided into basic divisions, each of which is closely monitored by an Assistant Project Officer.

Chemotherapy Research - Activities in this division are addressed to the exploration of new and potentially useful methodology for prediction, evaluation and improvement of drug therapy; the investigation of novel schedules of treatment; the investigation and application of predictability models for combination chemotherapy; and studies related to remission induction and maintenance with direct reference to problems and findings in clinical chemotherapy.

Drug Evaluation - Drug evaluation and biochemical pharmacology (drug metabolism) tasks conducted formerly under separate segments of this multifaceted contract have been consolidated under the Drug Evaluation Division. Emphasis is on the rapid screening of synthesis contractor analogs in in vivo assays designed specifically on the basis of the characteristics of the parent active agent; on the detailed evaluation of "active" drugs; on the evaluation of combinations of drugs of immediate clinical interest; and the study of the physiological disposition and metabolism of antitumor drugs and drug interactions, with special attention to therapeutic effectiveness and toxicity.

Immunology - The role of this division is to conduct basic and applied research in the area of tumor immunology which may lead to better procedures for controlling or suppressing tumor growth by the use of immunotherapy employed alone or in combination with antineoplastic drugs.

Organic Chemistry - This portion of the contract is directed to the synthesis of new compounds of potential usefulness, the examination of chemical problems of drug development, and studies on the structure of new agents.

Drug Distribution - This division provides services to the NCI for the storage and distribution of drugs and chemicals to various research laboratories throughout the country.

Toxicology - This portion of the contract was directed toward: (1) the study of the effect of oncolytic agents on the cardiovascular systems; (2) the mechanism of cardiotoxicity; (3) development of methods for the prevention or modification of the cardiotoxic effects, and (4) other pharmacodynamic studies of antineoplastic agents. This segment of the contract has been terminated during this Fiscal Year.

Laboratory Animal Science - The functions of this division are the development of new hybrids of inbred animals and the provision of holding facilities and animal caretaking services for the research studies conducted under the total contract.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM-92177)

The Contractor performs routine serologic assays for the determination of alloimmunization on NCI patients and potential platelet and leukocyte donors. Contractor serves as a serum repository for serialized serum specimens from all patients on the Pediatric Oncology Branch and has continued to perform well.

MIDWEST RESEARCH INSTITUTE (NO1-CM-12263)

This service-type contract was primarily concerned with the preclinical toxicologic evaluation of two potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies were directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity was characterized as to predictability, reversibility and schedule dependency of the toxicity. These characterizations were based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. All work was carried out in close cooperation with NCI and the results reported to the Laboratory of Toxicology. This contract has been terminated this Fiscal Year.

MIDWEST RESEARCH INSTITUTE (NO1-CM-33722)

Midwest Research Institute is one of the two facilities where analyses of experimental medicinal compounds and their clinical formulations are performed for Drug Research and Development. The Contractor determines the identity and purity of these materials, developing procedures for their analysis when standard methods are not available. The techniques employed include elemental analysis, chromotographic and spectrometric procedures, and other physiochemical procedures appropriate to the situation.

MIDWEST RESEARCH INSTITUTE (NO1-CM-33743)

Midwest Research Institute is one of three major synthetic contractors for Drug Research and Development. The primary objective of this contract is the rapid exploitation of leads generated by the Drug Evaluation Branch and clinical groups. This Contractor has provided a large number of compounds to DR&D for screening. These compounds represent the exploration of a variety of leads. Current investigation centers around the synthesis of (1) potential t-RNA o-methyl-transferase inhibitors, (2) receptor site-oriented antileukemic compounds based on the N-O-O triangulation hypothesis developed by this Contractor, (3) coralyne, nitidine, bleomycin and other natural products and their analogs, and (4) analogs of cytembena. The materials prepared have been of high purity, thoroughly characterized and in sufficient quantity for adequate evaluation.

MISSOURI, UNIVERSITY OF (NO1-CM-12323)

The objective of this contract is to develop procedures involving various advanced high resolution analytical techniques using specific instrumentation (e.g., GLC, amino acid analyzer, mass spectrometer, etc.) to measure quantitatively specific and potential biologic markers in the urine and blood of cancer patients and to utilize these procedures to study patients before, during and after therapy. Methods have been established for assay of polyamines (putrescine, spermidine, cadaverine and spermine) and methylated nucleic acid derivatives, as well as other minor nucleic acid bases in urine and clinical studies are in progress to determine the value of these markers for following disease status in patients. In addition, preliminary work has been initiated for the assay of several other potential markers in serum and urine including serum fucose/protein ratio, urinary β-aminoisobutyric acid, and hydroxyproline/creatinine ratio.

MONSANTO RESEARCH CORPORATION (NO1-CM-23715)

This service preparative contract provides for the synthesis of radioactive labeled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at Monsanto or acquired from other sources, are analyzed for purity and identity by radioautography, assay, etc. This contract also provides storage facilities for labeled materials and

MONSANTO RESEARCH CORPORATION (NO1-CM-23715) (CONTIN.)

distributes such as directed by the NCI staff.

MONSANTO RESEARCH CORPORATION (NO1-CM-33709)

This service preparative contract provides for the large-scale extraction, isolation and purification of natural products and the large-scale synthesis of compounds required for preclinical and clinical studies. The compounds prepared are not available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM-43703)

The objective of this contract is to identify the antitumor activity of new and old agents against advanced Stage II and IV ovarian carcinoma. To date, 14 patients have been randomized, 6 to Methotrexate as initial drug. None showed more than a 20% response of tumor size. Two patients crossed over to thioTEPA and had a complete regression. Eight patients were treated with thioTEPA as primary therapy. Five patients had a complete clinical response and three had less than 50% response.

MURPHY BREEDING LABORATORIES, INC. (NIH-73-C-868)

This procurement contract provides for the supply of 120,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for the Drug Research and Development compound evaluation program. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

NATIONAL ACADEMY OF SCIENCES (NO1-CM-40044 - TASK ORDER #6)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals; standards for nomenclature used to identify stocks and strains of laboratory animals; standards for methodology in maintaining axenic and gnotobiotic animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by *ad hoc* committees whose memberships represent commercial animal production colonies, governmental and academic institutions, commercial laboratories, and non-profit research institutions.

NATIONAL ACADEMY OF SCIENCES (NO1-CM-40044 - TASK ORDER #10)

This contract Task Order provides for conferences on diverse subjects that are intimately associated with the field of biomedical research. The agenda for specific meetings may be concerned with a common problem, or to develop the basis for a research program that is being considered by a segment of the scientific community. Conferences in the past have been held on research facilities; diseases of laboratory animals; professional training; the provision of animals for biological models for research; gnotobiotic technology; and requirements for standards in specific areas. Speakers are invited to present their thoughts, and time is provided for open forum discussions. The proceedings of these conferences are published and made available to the scientific community.

NEW JERSEY COLLEGE OF MEDICINE AND DENTISTRY (NO1-CM-23211)

This contract has as its major purpose serological diagnosis for the presence or absence of infectious mouse ectromelia, LCM, polyoma, M. hepatitis, M. adenovirus, PVM, Sendai, Theiler's (GDVII), K, Rheo 3, SV5, Toolan's H-1, and the Rauscher and Moloney viruses. The Contractor also carries out ancillary research concerned with the effects of viruses, such as vaccinia and polyoma, on the growth of tumors; interaction of polyoma virus with tumors; antibody-producing capacity of tumor-bearing mice and the effect of the route of injection on antibody levels; and a comparison of the sensitivity of caesarian-derived and other mice to polyoma virus. This activity furnishes information on the effects of "passenger" agents on the growth of tumors used in the screen and on their effects on drug action. During a contract period of one year, this laboratory performs approximately 65,000 rodent serum and 6,000 tumor fragment diagnostic tests. The Contractor also prepares large quantities of vaccinia virus used for immunizing mice against infectious ectromelia.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-02035)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The Contractor carries out the service by: (1) performing iso-antigenic typing tests of tumors used in the various screening tests, enabling Drug Research and Development to maintain and distribute tumors identical to the prototype; (2) developing techniques and material for antigenic typing of tumors new to the screening program, (3) determining strain specificity of tumors originating in the inbred mouse, and (4) performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic production centers.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-22072)

This contract provides support in the evaluation of the effects of chemotherapy on the growth and size of breast cancer lesions, toxicity and survival using mutually agreeable protocols. The working relationship is going well. A protocol comparing 5-Azacytidine with CCNU has been completed and overall incidence of tumor remissions induced by both drugs was lower than would have been expected from other standard agents such as 5-FU or from combinations. Forty-six patients have been entered into a protocol comparing Adriamycin to adrenalectomy and to a combination of Cytoxan, Fluorouracil and Prednisone (CFP). Thirty-two additional patients have been entered by Albany. Among the presently evaluable patients there are responses in 5/22 to Adriamycin, 11/25 to CFP, and 6/18 to adrenalectomy.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-33726)

The objective of this contract is the development of antiviral-antitumor agents from a class of ansa macrolide compounds, the streptovaricin complex. These agents were first shown by the Principal Investigator to be reverse transcriptase inhibitors and to interfere with viral replication. A significant finding has been their later observation of relatively selective *in vivo* effects, i.e., minimum cytotoxicity yet interference with viral replication. Attempts are underway to isolate the active component(s) from the crude complex.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-33734)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systemic and progressive understanding of the disease and promote its control. During the past contract year, new drugs and new drug combinations have been explored in patients with distant metastases.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-43714)

This contract is designed to undertake combined modality clinical studies in testicular tumors. The initial protocol treats Stage II nonseminomatous lesions with surgery followed by random allocation to radiotherapy, chemotherapy or radiotherapy plus chemotherapy.

NEW YORK UNIVERSITY MEDICAL CENTER (NO1-CM-71174)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and

NEW YORK UNIVERSITY MEDICAL CENTER (NO1-CM-71174) (CONTIN.)

control groups.

NOTRE DAME, UNIVERSITY OF (NO1-CM-23718)

Leukemia patients under treatment by chemotherapy are highly vulnerable to infection. Microorganisms that are part of the normal human flora may themselves cause serious infection. During the course of this investigation AKR mice have been successfully decontaminated and remained so when maintained under sterile conditions. These mice, when treated with immunosuppressive drugs, survived just as readily as do germ-free animals.

OHIO STATE UNIVERSITY (NO1-CM-33736)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therpy for lung cancer, and devise and implement therapeutic protocols that will lead to a systemic and progressive understanding of the disease and promote its control. During the past contract year, new drugs and new drug combinations have been explored in patients with distant metastases.

OHIO STATE UNIVERSITY (NO1-CM-71175)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

OREGON STATE UNIVERSITY (NO1-CM-23201)

The purpose of this contract is to acquire pharmacologic disposition information from animals so that such information is available prior to initiating clinical trials. Specifically, the three nitrosoureas, BCNU, CCNU and MeCCNU are being studied from two points of view. First, the complicated metabolic alterations these drugs undergo are being studied in detail. Second, the effects of these agents on the ability of the host hepatic microsomes to metabolize other drugs is under investigation.

PARKE, DAVIS & COMPANY (NO1-CM-23708)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. About 50% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities. This contract also provides for the large-scale extraction, isolation and purification of natural products required for preclinical and clinical studies.

PARKE, DAVIS & COMPANY (NO1-CM-43754)

This fermentation contract is designed primarily to obtain novel antitumor agents. This contract includes: (1) the preparation of fermentation beers from various microbes isolated from unique substrates and various parts of the world and fermented under a bevy of environmental and stress situations; (2) an *in vitro* tissue culture assay laboratory which will assist in pre-screening fermentation broths for cytotoxicity and will be used to help assay chemical fractions, fermentation improvement samples and large pilot plant lots more quickly; (3) a small biotransformation program; (4) the isolation work required to obtain the active component from the confirmed active beers; (5) the production of large quantities of antineoplastic agents approved for clinical trials; and (6) the preparation of appropriate dosage forms of such agents.

PENNSYLVANIA, UNIVERSITY OF (NO1-CM-12188)

The objective of this contract is to evaluate the effects of antitumor drugs on the electron transport processes in aerobic energy metabolism. The systems used are isolated heart and liver mitochondria, intact Ehrlich ascites cells and isolated perfused organs. Using O_2 electrode measurements of oxygen consumption as well as spectroscopic methods to determine the oxidation state of electron transport chromophores, an attempt is being made to identify effects which can be correlated with either the therapeutic activity or the limiting toxicities of antitumor drugs.

PENNSYLVANIA, UNIVERSITY OF (NO1-CM-33719)

This contract provides, on a multiphase basis, for implementing the design of and installing the Chemical Information System to replace the semiautomated system used to handle a wide range of compound accessioning activities. This effort will include: (1) writing, testing and documenting computer programs; (2) testing the system on a subsystem and total integrated basis; (3) converting existing and generating new data files; (4) preparing users' guides and training manuals, covering all aspects of system operation and management; (5) training operational and management staff; (6) installing the system; and (7) reviewing the performance of the new system.

This new system, upon completion, will provide improved capability to: (1) identify which compounds are new to the DR&D program, including the flagging of analogues and duplicates of compounds of interest; (2) produce computer-generated shipping documents and other communications to suppliers,

PENNSYLVANIA, UNIVERSITY OF (NO1-CM-33[illegible]3) (CONTIN.)

screeners, in-house staff, and contractors engaged in the accessioning process; (3) conduct on-line searches involving both structural and nonstructural information, using simplified inquiry procedures and providing structure output; (4) provide inventory control; and (5) maintain management, operational and quality control of the system.

PENNWALT CORPORATION (NO1-CM-12309)

The objective of this contract is the synthesis of coordination compounds. Few transition metal coordination compounds have been adequately investigated as potential antitumor drugs. This contract will synthesize coordination compounds of platinum, rhodium, nickel, cobalt and iron. This area of synthetic investigation may be expanded to include additional classes of metal complexes if screening test results warrant.

PHARM-ECO (NO1-CM-23707)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. About 50% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range.

PHILIPS ROXANE LABORATORIES, INC. (NO1-CM-12139)

This service contract provides the Division of Cancer Treatment with facilities for development, formulation and production of oral dosage forms of investigational drugs. The dosage forms prepared at Philips Roxane Laboratories are packaged, labeled and shipped to NCI for subsequent distribution to clinical investigators. This contract will expire May 1974.

POMONA COLLEGE (NO1-CM-33703)

This project has two major objectives. First is the attempt to derive a structure-activity correlation for anticancer drugs. This will be done using currently available data and the statistical methods of linear regression analysis. The second objective is the accumulation of fundamental data relating to hydrophobic bonding. These data will be used for further refinement of the structure-activity correlation as well as studies of the mechanisms of interaction of "mini-molecules" (drugs) with macromolecules (enzymes).

PUBLIC HEALTH SERVICE, DIVISION OF FEDERAL HEALTH PROGRAM SERVICES (TRANSFER OF FUNDS - Y01-CM-40003) (BALTIMORE, MARYLAND)

This Transfer of Funds permits service support by the hospital of NCI-intramural cancer chemotherapy programs at the USPHS Hospital in Baltimore, Maryland. Programs of clinical pharmacology, clinical use of the newer drugs for the treatment of cancer, and developments in improved patient care are on-going projects. Initial clinical trials of agents developed entirely within the NCI Division of Cancer Treatment Program and fundamental related laboratory research represent significant portions of the program.

RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK (N01-CM-33715)

This contract encompasses statistical and operational support for the Lung Working Party and includes activities conducted by the biostatistician and by the Operations Office of a multidisciplinary, cooperative study group. Additional responsibilities include central randomization and stratification of patients who enter studies and preparation and monitoring of monthly fiscal reports for the members of the Working Party, progress reports by membership institutions every four months and annual reports. Progress has been shown during the past contract year.

RESEARCH TRIANGLE INSTITUTE (N01-CM-92019)

The primary purpose of this contract is to fractionate confirmed active plant extracts in an attempt to isolate and identify the active antineoplastic compound. In addition, active fermentation materials obtained from other contractors are isolated, purified and identified. The plants used for this work are primarily obtained from the U. S. Department of Agriculture. The Contractor has many plants which are or have been in some stage of fractionation and several fermentation crude materials which are being purified. Several of these active agents have been isolated and are being evaluated more broadly. A KB cell culture facility is operational and used to expedite fractionation work.

RHODE ISLAND, UNIVERSITY OF (N01-CM-12312)

This contract provides for the synthesis of nitrogen heterocyclic compounds with potential antitumor properties. The compounds being synthesized are analogs and derivatives of the imidazolopyrazine and isoxazolopyrazine ring systems. Both systems have structural similarity to purines and have the possibility of interfering with normal purine metabolism.

SCRIPPS CLINIC AND RESEARCH FOUNDATION (N01-CM-22042)

The objective of this contract is to prepare and test analogs of the coenzyme form of vitamin B_{12}. These are to consist of alterations in both base and/or sugar of the adenosine portion of the molecule. Synthesis will be either enzymatic or chemical. After establishing identity of the agents, they are

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NO1-CM-22042) (CONTIN.)

tested first for cell penetration and anti-B_{12} activity in vitro. Potentially useful agents are then to be made in larger quantities for in vivo testing of anticancer activity.

SHEEHAN INSTITUTE FOR RESEARCH (NO1-CM-23719)

The objective of this contract is the synthesis of new compounds with potential as Central Nervous System (CNS) antitumor agents. Specifically, compounds related to ergocornine, which are CNS active and which may pass the blood-brain barrier, are synthesized and evaluated in appropriate intraperitoneal and intracerebral test systems. Target directed CNS drugs utilizing the carrier principle are also designed and synthesized.

SIMONSEN LABORATORIES, INC. (NO1-CM-12101)

The Contractor maintains a genetic production colony of six inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production propagation.

SIMONSEN LABORATORIES, INC. (NO1-CM-23219)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SIMONSEN LABORATORIES, INC. (NIH-73-C-816)

This contract furnishes approximately 409,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

SMALL BUSINESS ADMINISTRATION (NO1-CM-43719)

The purposes of this contract, which was initiated this year, are to provide supportive services in immunology, virology and tissue culture to the cancer chemotherapy program. The immediate goals are: (1) to develop a sensitive in situ immunological assay system for detecting virus-related antigens in human leukemic cells as a potential diagnostic tool, (2) to extend and quantitate the effect of Rifamycin SV derivatives on reverse transcriptase and in preventing cell transformation in vitro, (3) to evaluate the in vivo metabolism of Rifamycin SV derivatives, and (4) to perform specialized cloning experiments of human leukemic cells and other human tumor cells of interest.

SOUTH SHORE ANALYTICAL AND RESEARCH LABORATORIES, INC. (NO1-CM-40932)

This service-type contract was primarily concerned with the preclinical toxicologic evaluation of two/three potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies were directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity was characterized as to predictability, reversibility and schedule dependency. These characterizations were based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced deaths. Special investigations of drug-induced toxicity were conducted at the request of the Project Officer. All work was carried out in close cooperation with NCI and the results reported to the Laboratory of Toxicology. This contract has been terminated this Fiscal Year.

SOUTHERN ANIMAL FARMS (NO1-CM-22013)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SOUTHERN ANIMAL FARMS (NIH-73-C-863)

This contract furnishes approximately 409,000 B6D2F_1 (57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CM-23241)

This contract was instituted to define the pharmacokinetics of adriamycin and guanazole in man. Initial efforts have been directed at defining the metabolism and distribution of adriamycin in rabbits in order to develop a comprehensive pharmacokinetic model which can then be applied to man. The initial phases of this study have been completed, and the application of a theoretical model, based on the findings in the rabbit, to the clinical stituation, is the goal of work in the coming year.

SOUTHERN RESEARCH INSTITUTE (NO1-CM-02083)

The principal objective of this contract is to compare the relative toxicity and therapeutic activities of cancer chemotherapeutic agents alone and in combination when different dosage regimens are used in rodent tumor systems and in non-human primates or dogs. These studies are intended to serve as a basis for the design of a combination drug regimen acceptable for clinical trial. The combinations of adriamycin-cyclophosphamide and 5-fluorouracil-melphalan are currently under study.

SOUTHERN RESEARCH INSTITUTE (NO1-CM-12098)

Objectives of this comprehensive research and development contract are: (1) development of biological principles leading to discovery of more effective anticancer therapy, (2) development of improved assays for predicting clinical usefulness, (3) detailed evaluation of new drugs as potential clinical candidates, and (4) primary screening *in vivo* and *in vitro*. Objectives are realized through (a) basic studies of factors influencing growth spontaneous and transplated animal tumors, (b) studies of the dynamics of tumor cell proliferation and the effects of drugs thereon, and (c) quantitation of drug activity relative to factors which influence it (e.g., treatment route and schedule). Studies of drug combinations and studies of combined treatment modalities are conducted to uncover superior therapies leading to cures of animal tumors. Mammalian cell culture research and development is aimed at defining the influence of drug concentration and duration of effect at the target cell, and the cell cycle stage specificity of active drugs. Antimicrobial testing is aimed at providing information relative to mechanisms of drug action and at identifying suitable organisms for bioassay of effective drug levels in body fluids.

SOUTHERN RESEARCH INSTITUTE (NO1-CM-12189)

The purpose of this contract was to study the effect of potential anticancer agents on cell membrane formation, with the hope of learning whether such inhibition can be a cause of cell death. This was approached by determining the effects of these drugs on incorporation of labeled precursors into membrane constituents. The contract was terminated in December 1973.

SOUTHERN RESEARCH INSTITUTE (NO1-CM-33712)

Southern Research Institute is one of the basic synthesis contractors for Drug Research and Development. The chemistry program is oriented to the biological and biochemical rationales developed in other programs, and is integrated with the screening, therapeutic and biochemical activities at Southern Research Institute. The areas of investigation include: purine antimetabolites, including 9-substituted-9H-purines, ring analogs of purines, nucleotides, and nucleosides; folic acid antagonists, directed toward the preparation of "simpler" derivatives and variants of the methotrexate and aminopterin structures; imidazole antimetabolites and analogs of imidazoles; *N*-nitrosourea analogs; aryl and heterocyclic triazenes; metabolites of cytoxan; and compounds containing very reactive chemical moieties.

SOUTHERN RESEARCH INSTITUTE (NO1-CM-50654)

The purpose of this contract is to obtain basic quantitative pharmacologic information in animals which will indicate the requirements of drug dosage and scheduling necessary to give maximum tumor cell kill while minimizing toxicity to the host's normal cells. This involves the development and application of highly sensitive analytical methodology to measure drugs and their metabolites

SOUTHERN RESEARCH INSTITUTE (NO1-CM-50654) (CONTIN.)

in body fluids and tissues and the estimation of half-lives and cumulative CXt values in animals and man. During the past year, studies have involved cyclocytidine, 6-thioguanine, and arabinosylcytosine, actinomycin-D, hexamethylmelamine and the development of numerous assays for antitumor agents in varying stages of advancement.

SOUTHERN RESEARCH INSTITUTE (NO1-CM-60029)

The major objective of this contract is to develop basic biochemical knowledge useful in the design of new drugs or in the more effective use of known agents. To accomplish this aim, studies are underway on: (1) sites of action and the metabolism of compounds with antitumor activity; (2) the biochemical basis for resistance to important antitumor agents; and (3) the comparative biochemistry of tumors and various normal cells.

SPARTAN RESEARCH ANIMALS, INC. (NIH-73-C-858)

This procurement contract provides for the supply of 104,000 $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice for the Drug Research and Development compound evaluation program. Breeding animals originate in genetic centers.

SPARTAN RESEARCH ANIMALS, INC. (NIH-73-C-878)

This procurement contract provides for the supply of 104,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for the Drug Research and Development compound evaluation program. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

STANFORD RESEARCH INSTITUTE (NO1-CM-12162)

This service preparative contract provides for the synthesis of radiolabeled chemicals and drugs for use in preclinical pharmacologic and clinical studies. Many of the materials prepared are not available from commercial sources and are synthesized. All materials, whether prepared by the contract group or acquired from other sources, are analyzed for purity and identity by radioautography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the NCI staff.

STANFORD RESEARCH INSTITUTE (NO1-CM-23200)

The purpose of this contract is to acquire pharmacologic disposition information from animals so that such information is available prior to initiating clinical trials. The following investigations are conducted: (1) plasma levels and urinary, biliary and fecal excretion rates of drugs and/or metabolites of compounds of interest to the chemotherapy program; (2) tissue distribution of

STANFORD RESEARCH INSTITUTE (NO1-CM-23200) (CONTIN.)

the parent drug and metabolites after oral and parenteral administration; (3) isolation and identification of the major drug metabolites; and (4) properties of the enzyme systems responsible for metabolism (subcellular localization, cofactor requirements and susceptibility to known inducers and inhibitors of drug metabolism). Both *in vivo* and *in vitro* studies are conducted using at least three animal species, including one species of subhuman primates.

STANFORD RESEARCH INSTITUTE (NO1-CM-33723)

This contract is concerned with quality control studies on compounds prepared for preclinical toxicology and clinical trial. The Contractor evaluates bulk chemicals and formulation drugs as to their identity, assay and purity, to provide assurance of high quality of the necessary analytical data for the Food and Drug Administration. This area of work provides elemental analysis, as well as the more sophisticated techniques such as chromatography (paper, thin layer, liquid and gas), absorption spectroscopy (ultraviolet, visible and infrared), nuclear magnetic resonance, and other physical-chemical procedures appropriate for the situation. In some cases, methods of assay must be developed for new structures in order to maintain adequate surveillance on the quality of all drugs used in clinical trials.

STANFORD RESEARCH INSTITUTE (NO1-CM-33742)

Stanford Research Institute is one of the three major contractors devoted to the synthesis of new chemical structures. The research program encompasses such areas as nucleosides, antimetabolites, antibiotics, alkaloids and other areas as may be requested.

The completion of the synthesis of formycin B has opened the path for the synthesis of additional pyrazolo[4,3-*d*]pyrimidine C-nucleosides. Exploitation of leads in the indole alkaloid field is being pursued by synthesis of new indole alkaloids. Compounds related to the known indole alkaloids, ellipticine and olivacine, are under investigation. Special attention is being directed to the study of the chemistry of the antibiotics daunomycin and adriamycin in order to develop agents of greater specificity at the molecular level. Analogs of daunomycin and adriamycin are being prepared and a total synthesis of adriamycin is under investigation. A modest effort is being extended toward preparing simplified synthetic daunomycin analogs that retain only major features of the parent molecule. Also under investigation, is the synthesis of a group of compounds designed to intercalate into DNA in the manner proposed for several antitumor compounds and some unusual heterocyclics.

STARKS ASSOCIATES, INC. (NO1-CM-23203)

This service preparative contract supplies the Drug Development Branch with compounds required for drug evaluation studies, with 40% of the effort being devoted to the preparation of clinical materials. Preparations vary in quantity

STARKS ASSOCIATES, INC. (NO1-CM-23203) (CONTIN.)

from 5 grams to multikilograms.

STARKS, C. P., INC. (NO1-CM-23705)

This is a service contract which supports the Drug Development Branch's efforts in the procurement of chemicals and drugs for the evaluation in the primary screen. The Contractor is required to: (1) provide liaison services to develop new sources of material and to encourage potential suppliers to participate in the program through voluntary contribution of samples; (2) collect compounds; (3) provide support services in the processing of compounds; and (4) aid in the development of methods for selecting materials for the screen.

TACONIC FARMS, INC. (NIH-73-C-912)

This contract furnishes approximately 295,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

TECHNICON INSTRUMENTS CORPORATION (NO1-CM-33746)

The purpose of this contract is to develop equipment for the rapid, alternated individual determination of polyamines (spermidine, spermine and putrescine) in urine, whole blood, plasma or serum. This equipment will be designed to determine the sum of the polyamines in any of the above-mentioned biological fluids.

TENNESSEE, UNIVERSITY OF (NO1-CM-02084)

The objectives of this contract mainly include formulation development studies and small batch production of oral solid dosage forms. The Contractor also has the capabilities to prepare parenteral dosage forms in pilot batch lots. All work is assigned by the NCI.

TEXAS INBRED MICE COMPANY (NO1-CM-43711) (FORMERLY PH43-NCI-C-68-88)

This rodent production center supplies a variety of inbred and hybrid rodents for tumor transplantation; for hybrid production (at Texas Inbred and other animal production contractors); and for compound evaluation studies. Animals are supplied from a colony of 25 strains of rodents.

TEXAS, UNIVERSITY OF (NO1-CM-12094)

This contract was designed to assess the value of protected environments in the chemotherapy of acute leukemia using appropriate controls for a quantitative and qualitative comparison. In this study, a protected environment is defined as a laminar-air-flow room, used in conjunction with an extensive antimicrobial prophylactic regimen developed from previous studies. This contract is in the process of renewal after recompetition.

TEXAS, UNIVERSITY OF (NO1-CM-33710)

The objective of this contract is to identify clinical effectiveness of antitumor agents for the treatment of advanced ovarian carcinoma. This common tumor of women is known to be quite sensitive to a variety of alkylating agents as a class. The Contractor will randomize 100 previously untreated patients with Stage II or IV ovarian carcinoma to one of three choices of treatment. Melphalan, 5-FU and Hexamethylmelamine studies are nearly completed. All three drugs show significant activity. New studies with Adriamycin and combinations are being initiated in the next contract year.

TEXAS, UNIVERSITY OF (NO1-CM-33737)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systemic and progressive understanding of the disease and promote its control. During the past contract year, new drugs and new drug combinations have been explored in patients with distant metastases.

TEXAS, UNIVERSITY OF (NO1-CM-61156)

The purpose of this contract is to carry out studies on the pharmacology and physiological disposition of new antitumor agents under development by the National Cancer Institute. During the current year, studies have been carried out on aspects of the pharmacology of the following agents: cyclocytidine, palmo-ara-C, benzoyl ara-C, Asaley, ara-adenine, ara-6-mercaptopurine, 6-thioguanine and the Yoshi dimethane-sulfonate compound, bis (2-methylsulfonoxypropyl)amine (NSC-102627). A cellular pharmacology program has been initiated, utilizing human melanoma and lymphoma cell lines. The effects of antitumor agents are being evaluated in terms of parameters such as cell cycle stage sensitivity, cell kill as a function of duration of treatment, and repair of drug-induced injury. Studies under this contract are carried out in experimental animals and in cell culture lines, but are closely interrelated with Phase I clinical trials and clinical pharmacologic studies conducted at the M. D. Anderson Hospital and Tumor Institute, and elsewhere.

TRACOR JITCO, INC. (NO1-CM-33744)

The purpose of this contract is twofold: (1) it provides the programming needed to run the Drug Distribution and Cost Reporting System and the Clinical Drug Inventory, and (2) it provides the resources needed to maintain the files of the Drug Distribution and Cost Reporting System. These files serve as a record of all drugs sent to investigators by the NCI and include such information as drug name, NSC number, lot number, formulation, quantity shipped, shipment cost, investigator name, address and group affiliation. Data are derived from bulk chemical and pharmaceutical receipt records and from clinical drug request forms.

TRW, INC. (NO1-CM-12190)

The purpose of this contract is to evaluate the potential use of selected chemotherapeutic agents which may be used intrathecally. This toxicologic evaluation is based upon activity observation, hematology, clinical chemistry, gross pathology, light and electron microscopic examination of animals which have received graded doses of specific chemotherapeutic agents intrathecally. These studies are carried out in close cooperation with the Baltimore Cancer Research Center personnel for purposes of monitoring drug selection and results.

TULANE UNIVERSITY (NO1-CM-33701)

This primary genetic center serves to establish foundation colonies of caesarian-derived inbred rats (strains M520, OM/N, Fischer 344, Buffalo, W/L, ALB, ACI and other inbred strains as required). Its objectives are to improve the quality and maintain the genetic characteristics of the strains for program activities. Foundation colonies are propagated in germ-free isolators, and small-scale production colonies are housed in bio-containment environments.

UNIVERSITY HOSPITAL (NO1-CM-33725)

The purpose of this contract is to evaluate potential new biological markers in patients with pancreatic cancer. Patients will be treated with chemotherapy or surgery and/or radiation therapy as indicated. Sequential measurements are performed for plasma levels of CEA and cyclid AMP and other potential markers. A Phase II study of adriamycin in patients with advanced disease will be performed.

UPJOHN COMPANY (NO1-CM-23709)

The objective of this contract is the synthesis of analogs and derivatives of the clinically effective agent, streptozotocin. The major emphasis is on preparing analogs with modifications in the sugar portion of the molecule. Specifically, the Contractor is preparing nitrosomethylureido derivatives of some hexoses, pentoses, tetroses, disaccharides, lactones, glycitols and nucleosides. This contract will terminate in May 1974.

UPJOHN COMPANY (NO1-CM-43753)

This contract has for its primary objective the research and development of potentially useful antineoplastic agents from fungi, bacteria and actinomycetes fermentation beers, including isolation, purification, characterization, production, biochemistry and pharmacology efforts.

The Upjohn Company has played a leading role in the development and exploitation of cell culture methods, both for assays on fractionation samples and for primary screening. Leads developed from this screen are being tested in-house in the leukemia *in vivo* screen and actives are given top priority for chemical fractionation.

Major efforts in this contract are also devoted to biotransformation of known Upjohn antibiotics, studies on the mechanism of action and physiological disposition of clinical drugs developed within the contract program and phases of toxicological and pharmaceutical studies within the capability of the Contractor and requested by NCI.

UTAH, UNIVERSITY OF (NO1-CM-23710)

This contract is for the synthesis of nucleoside analogs and derivatives, including nucleoside antibiotics, for screening as antitumor agents. The compounds selected for synthesis are based upon biological and biochemical rationale. The areas of investigation include: (1) analogs of the antibiotics, tubercidin, sangivamycin and toyocamycin and other nucleoside antibiotics, (2) derivatives of the C-nucleoside formycin, (3) pyrazolo[4,3-*d*]pyrimidine and pyrazolo[3,4-*d*]pyrimidine nucleosides, (4) imidazo[4,5-*c*]pyridine, imidazo[4,5-*b*]pyridine and *v*-triazolo[4,5-*c*]pyridine nucleosides, and (5) imidazole and benzimidazole nucleosides. This Contractor provides these difficult materials in quantities sufficient for *in vivo* antitumor evaluation by methods of synthesis developed by this group.

VALUE ENGINEERING COMPANY (NO1-CM-33706)

Data processing services are provided by this contract to Drug Research and Development. The main areas of emphasis are: (1) reducing and disseminating information developed in the screening program of the Drug Evaluation Branch, DR&D (including special queries of the master files via computer); (2) documenting all programs and contractor's procedures for data handling and running computer programs; (3) maintaining the computer programs so that they are able to run in the Division of Computer Research and Technology environment; (4) reducing and disseminating information on the receipt of compounds including those of particular interest to the Division of Cancer Treatment program; (5) expanding the present system to incorporate secondary testing not previously stored on the master files; (6) redesigning the existing system and developing new formats and methods of data collection; (7) providing instructions for screening laboratories and suppliers of materials relating to collection and dissemination of data.

VERMONT, UNIVERSITY OF (N01-CM-33716)

This contract provides for the extension of the Computerized Problem-Oriented Medical Information System to the Cancer Treatment area by integrating structured input ("cancer displays") into the system, thereby coupling learning with doing for all personnel who care for individual cancer patients entered on problem-specific plans (research protocols).

VETERANS ADMINISTRATION (NCI-CR-(64)-4)

This program is a cooperative venture between the Veterans Administration and the National Cancer Institute for the clinical evaluation of potential anticancer agents. It is to continue along the same lines as previously.

VIRGINIA, UNIVERSITY OF (N01-CM-12099)

This contract provides for the fractionation and isolation of active agents from plants with confirmed antitumor activity which stem from the program for screening of crude extracts and are assigned to contract chemists. During the current report period, a number of crystalline materials were isolated and are now undergoing further study at various stages of evaluation.

WALTER REED ARMY MEDICAL CENTER (Y01-CM-40005)

The primary objective of this interagency agreement is to provide support for Walter Reed Army Medical Center, Hematology-Oncology Service. This includes collaborative studies with Acute Leukemia Group B and the Medical Oncology area. Protocols are available for patients with acute lymphocytic leukemia, acute granulocytic leukemia, chronic lymphocytic and chronic granulocytic leukemia, Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma, and brain, head and neck, breast and testicular tumors. Accrual to the protocols has been satisfactory.

WARF INSTITUTE, INC. (N01-CM-43707)

This represents a service contract to prepare 50% aqueous-ethanol extracts of plant material at the rate of approximately 11,000 extracts per year. A semi-quantitative test for alkaloids and tannins is run on plant materials of sufficient interest to be considered for fractionation. The majority of the Contractor's plants are obtained through a Transfer of Funds agreement between the NCI and the U. S. Department of Agriculture. During the year, the special study of the extraction procedure was continued involving the preliminary fractionation, on a trial basis, of 500 plants in order to develop a procedure for increasing the yield of L1210 active plants.

WARF INSTITUTE, INC. (NO1-CM-60-94)

This is a service contract for the in vivo screening of new, crude plant extracts, fractionations of previously confirmed natural products, new synthetic materials, and follow-up testing of active materials. Currently, testing is being conducted at a level of approximately 50,000 tests per year according to DR&D protocols. The P388 lymphocytic leukemia is employed as the test system for crude natural products. Those that are active in this system are then tested in the L1210 leukemia. The L1210 leukemia is utilized for the testing of new synthetic materials. Other DR&D tumor systems may occasionally be used in the further testing of materials that were active in these systems or for analogs of materials that were active in these systems. Schedule dependency studies are conducted upon request for materials of potential clinical interest. Methodology and/or research and development, as well as special research programs are scheduled as the need arises and are directed by the Project Officer.

WASHINGTON HOSPITAL CENTER (NO1-CM-43710)

The objectives of this new contract are to label bleomycin with gamma-emitting isotopes for external counting, and to study the tumor localizing properties of this material for staging and diagnostic evaluation. Material has been shown, in a preliminary manner, to localize in a higher proportion of tumors than other isotopes such as Gallium-67. The ability to identify tumor foci in the body will allow for more precise staging evaluation as well as for following changes in tumor size after treatment.

WASHINGTON STATE UNIVERSITY (NO1-CM-22059)

This primary genetic center has as its objectives the development of germ-free foundation colonies of inbred rodents. Pedigreed animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artifically contaminated with pure cultures of non-pathogenic organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to secondary genetic centers, which in turn produce breeding animals for large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small-scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies.

WASHINGTON, UNIVERSITY OF (NO1-CM-12193)

The purpose of this research-type contract was to explore the effects of high doses of chemotherapeutic agents in conjunction with autologous and homologous bone marrow infusions. Emphasis was directed to the hematopoietic toxicity and immunosuppressive manifestations of the agents. The investigation involved determining which antineoplastic agents or combinations of agents have dose limiting bone marrow toxicity and in fact do not produce significant immunosuppression. In addition, these studies were intended to reveal any unexpected drug-induced toxicity associated with unusually high doses of selected oncolytic drugs. This contract has been terminated this Fiscal Year.

WEIZMANN INSTITUTE OF SCIENCE (PL-480 - AGREEMENT NO. NIH-06-025-1)

The objectives of this contract are: (1) to seek selectivity in cancer therapy through modification of the physical properties of anticancer agents by conjugation with various oligomers and polymers, and (2) to produce polymers of amino acids which in themselves may have anticancer activity.

WISCONSIN, UNIVERSITY OF (N01-CM-12275)

The principal objective of this contract is to study the mechanism of replication of genetic information, contained by RNA-tumor viruses, as well as the mechanism of integration of this information into the host chromosome. To accomplish this objective, studies are in progress with purified enzymes (from avian myeloblastosis virus) in order to understand clearly the enzymic reactions involved. The results on each isolated reaction will be assessed with relation to the physiology and life cycle of the intact virus. The DNA and RNA enzymes have been implicated in the etiology of human cancer. An understanding of the properties and mechanisms of these enzymes is, therefore, fundamental for the eventual solution of this problem. In addition, RNA-DNA hybrid template-primers of defined sequence and purity will be prepared and provided to the Project Officer.

WISCONSIN, UNIVERSITY OF (N01-CM-33733)

This contract, utilizing cell culture techniques, provides a rapid assay as an aid in the purification of potential antitumor agents from plant materials. The materials tested are the fractionation products prepared for the purpose of isolating the active agents from materials having established antitumor activity in the DR&D *in vivo* screen. The principal methods used are an agar diffusion system and bioautography of paper chromatograms.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM-12339)

The objective of this contract is to develop anticancer agents which prevent vital cell function or replication by intercalating into nucleic acids to prevent strand separation. Evaluation of the strength of binding is made by determination of melting temperature and calculation of binding constant. Various types of nucleic acid templates are used, such as deoxy-deoxy, ribo-ribo, and hybrid deoxy-ribo strands. Analogs of a parent compound are made to increase desirable characteristics and minimize unfavorable ones. *In vitro* cytotoxicity and *in vivo* antitumor screening of the agents is done.

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1-CM-33711)

The objective of this contract is to provide *in vitro* screening systems to select promising anticancer agents of the antifolate type. The compounds are assayed for inhibition of dihydrofolate reductase from L1210 leukemia cells, from human chronic myelogenous leukemia and from normal red blood cells. Special pharmacologic studies on selected agents are also carried out.

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1-CM-33740)

This service-type contract was concerned primarily with the preclinical toxicologic evaluation of two potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies were directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity was characterized as to predictability, reversibility and schedule dependency. These characterizations were based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. All work was carried out in close cooperation with NCI and the results reported to the Laboratory of Toxicology. This contract has been terminated this Fiscal Year.

Serial No. 3409
1. Office of Deputy Director
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Leukocyte Kinetics and Characteristics of Cell Proliferation

Prinicipal Investigators: Gregory Sarna, M.D. and Ronald Barr, M.R.C.P.
M.R.C. Path.

Other Investigators: Seymour Perry, M.D. and Jacqueline Whang Peng, M.D.

Man Years:

Total:	3.1
Professional:	2.1
Other:	1.0

A. Objectives

1. To evaluate the cytokinetic characteristics of human acute leukemic blast cells, both in the untreated state and as perturbed by chemotherapy, in order to gain information of potential value in planning and scheduling chemotherapy.

2. To study the characteristics of the various phases of the cell cycle of tumor cells from the AKR mouse leukemia model with emphasis on the resting (G_o) cell.

a. Studies of cytokinetic characteristics -- chemotherapeutic effects and clonogenic potential.

b. Studies of biochemical characteristics

3. To gain insight into normal leukocyte and stem cell kinetics.

B. Methods Employed:

1. Leukemic cells, either human or murine, are purified and separated as to size by the 1-g sucrose gradient technique. Separated cells are studied for characteristics such as uptake of tritiated thymidine, DNA content, clonogenicity in a transplant recipient (murine) or in a diffusion chamber (murine, intraperitoneal), and various biochemical characteristics (e.g., polyamine content, terminal transferase content, DNA polyamerase content). These characteristics may be studied as a function of preceding chemotherapy and may be compared to similar characteristics in non-leukemic cell

2. Normal human peripheral blood cells are studied as to clonogenicity of unseparated cells and of cells separated into groups according to size by the 1g sucrose gradient technique. Clonogenicity of the resultant fractions is assayed either by growth in a peritoneal diffusion chamber (in collaboration with Dr. Jacqueline Whang-Peng) or in semi-solid conditioned medium (in collaboration with Dr. Joan Bull).

C. Major Findings

1. Human Acute Leukemia

A. Blasts from acute myelogenous leukemia (AML) are generally larger and more variable in size than blasts from acute lymphoblastic leukemia (ALL).

B. Absolute blast cell size does not directly relate to blast proliferative activity.

C. Relative cell size, within a given patient's population of blast cells, does relate to proliferative activity. The large cells and some medium cells tend to be in S, G_2 or M phases of the cell cycle. The small and some medium cells tend to be in G_1 or G_0 phase. Increasing cell size correlated with progress through the cell cycle.

D. In a patient with AML, cytosine arabinoside and 6-Thioguanine given on a q12 hours schedule, ceased a progressive depletion of leukemic blasts. Relatively large blasts were destroyed in a cycle specific manner. Small blasts were recruited into cycle (an effect most marked at 1.5 days) and then destroyed.

E. In a patient with ALL, L-asparaginase resulted in a cycle nonspecific loss of both large and small cells as well as a cycle specific loss of large cells and medium cells. Recruitment and synchronization were not observed. The data are consistent with L-asparaginase causing a "G_1/S block" and arrest in blasts prior to entry into S phase.

F. In the leukemic phase of Burkitt's lymphoma, cell size seems to correlate with proliferative capacity.

2. AKR mouse leukemia

A. Cytokinetic

1. The effects on the cell cycle of various chemotherapeutic agents were studied. Data are not yet complete but preliminary interpretation suggests

Cis - Platinum, adriamycin, daunomycin, L-asparaginase and actinomycin D all selectively spare small cells. Daunomycin and Actinomycin D showed a tendency to spare medium cells as well.

B. Clonogencity

1. Surviving small, medium, and large cells had significant clonogenic potential as measured by AKR mouse 1st passage assay (with the death from secondary leukemia in 3 months as an endpoint) after treatment with L-asparaginase. Clonogenic potential was decreased, particularly in the small cell fractions with the other drugs tested. This would be compatible with L-asparaginase having a significant cytostatic effect as well as cytocidal effect.

2. Clonogenic potential of untreated cells using the *in vitro* peritoneal chamber approach, in collaboration with Dr. Whang Peng, has shown a limited but definite potential for untreated unseparated AKR leukemia cells to form macrophages and atypical lymphs in up to two weeks of culture. Cells separated as to size grew less well regardless of size. These data are preliminary and longer term cultures are underway.

C. Biochemical

1. In collaboration with Dr. Olle Heby *et al*, measurement of polyamine (putrescine, spermadine, spermine) concentrations within AKR leukemic cells at different stages of the cell cycle demonstrated a higher concentration of spermine in G_0 than in G_1, and a progressive and sequential increase of putrescine, spermidine and spermine as cells progressed from G_1 to M.

Orderly synthesis of polyamines was also noted in normal AKR thymus cells, although lower levels of polyamines were present. This data is of significance in relationship to possible techniques for distinquishing resting from actively cycling neoplastic cells and for elucidating the role of polyamines as biochemical tumor markers.

2. Terminal transferase -- In collaboration with Dr. Ronald McCaffrey, terminal transferase, a thymus specific DNA polymerase which has been found as a marker in some cases of human acute lymphoblastic leukemias, has been demonstrated in AKR leukemic spleens and lymph nodes as well as thymuses. Work is under way at present to see whether or not presence or concentration is a function of cell cycle stage.

3. Human stem cell kinetics -- Preliminary work is being done involving cultures of size dependent subgroups of human peripheral blood lymphocytes by the conditioned medium and peritoneal chamber techniques. Data are too preliminary to report.

D. Proposed Course of Project

1. Studies of human tumor kinetics will be further studied using the cytokinetic techniques previously described.

A. *In vivo* studies of leukemia kinetics, using pulse and continuous thymidine labeling, will be performed on suitable patients.

B. Techniques for processing and studying pediatric solid tumors will be expanded and utilized.

2. Studies into human stem cell kinetics and AKR clonogenicity will be continued.

3. Biochemical approaches to characterizing various phases of the cell cycle will be expanded.

E. Honors and Awards

1. Sarna, G., Omine, M., Perry, S.: Cytokinetic Characterization of Human Acute Leukemia Before and After Treatment. Presented at the Annual Meeting, American Society of Hematology, Chicago, Illinois Dec. 1973.

2. Perry, S. Introductory remarks on cell kinetics and prognosis. Workshop on "Prognostic Factors in Human Acute Leukemia" Ulm, Germany.

3. Sarna, G., Omine, M., Perry, S.: Cytokinetics of Human Acute Leukemia and Effects of Chemotherapy. Presented at the 9th Annual Joint Meeting, Professional Associations of the U.S. Public Health Service, J.D. Lane Award Finalist.

PUBLICATIONS:

Omine, M., Sarna, G., and Perry, S.: Composition of Leukemic Cell Populations in AKR Leukemia and Effects of Chemotherapy. Europ. J. of Cancer. 9: 557-565, 1973.

Heby, O., Sarna, G.P., Marton, L.J., Omine, M., Perry, S., and Russell, D.H. Polyamine Content of AKR Leukemic Cells in Relation to the Cell Cycle. Cancer Res. 33: 2959-2964, 1973.

Perry, S. and Omine, M.: Perturbation of Leukemic Cell Population in AKR Mice Due to Chemotherapy. Cancer Res. 33: 2596-2602, 1973.

Heby, O., Sarna, G., Marton, L.J., Omine, M., Perry, S., and Russell, D.H.: Polyamine Content of AKR Leukemia Cells in Relationship to Cell Cycle as Reflected by 1 x g Sucrose Density Gradient Separation. Presented at the 8th Congress of the Nordic Society for Cell Biology, Oslo. Published in Norwegian Journal of Zoology, July 1973.

Sarna, G., Omine, M. and Perry, S.: Cytokinetic Characterization of Human Acute Leukemia Before and After Treatment. Blood, Vol. 42: 1021 (Dec.) 73.

Perry, S. and Spivak, J.L.: Evaluation of ^{51}Cr as a Leucocyte Label. British Journal of Haematology. 25: 321-330, 1973.

Perry, S.: Cancer Chemotherapy: A Broad Overview in Proceedings, Seventh National Cancer Conference. J. B. Lippincott Co., Phila. pgs. 103-108, (Sept.) 1972.

Guerry, D., Dale, D. C., Omine, M., Perry, S., and Wolff, S.M. Periodic Hematopoiesis in Human Cyclic Neutropenia. J. Clin. Invest. 52: 3212-3220, 1973.

Perry, S.: Summation and General Discussion, Selman A. Waksman Conference on Actinomycins: Their Potential for Cancer Chemotherapy. Children's Cancer Research Foundation. Boston, February 5 and 6, 1973. Cancer Chemotherapy Reports. 58: (1) 117-120, (Jan./Feb.) 1974.

Perry, S.: Introduction to Nomenclature and Classification of Acute Leukemias. Recent Results in Cancer Research. G. Mathe, P. Pouillart, and L. Schwarzenberg, (Eds.), Berlin, Springer-Verlag. 43: 1-5, 1973.

Perry, S.: Conclusions of the Working Session on Nomenclature and Classification. 43: 105-106, 1973. Recent Results in Cancer Research. G. Mathe, P. Pouillart, and L. Schwarzenberg, (Eds.) Berlin, Springer-Verlag. 43: 105-106, 1973.

Serial No. NCI-6401
1. Office of the Deputy
Director
2. Bethesda, Maryland

PHS-NIH
Individual Project Reports
July 1, 1973 Through June 30, 1974

Project Title: (A) Data Processing Systems for Scientific and Management Information in the Division of Cancer Treatment
(B) Simulation Study of the Preclinical Development of Anticancer Drugs

Principle Investigator: Meyer H. Gordon

Other Investigators: NCI: Barry Goldfarb, Anthony Kontaratos
TRW: Gerald E. Stock
SKF: Edmund B. Pyle

Cooperating Unit: DMB, DCRT

Man Years:

Total: 2.0
Professional: 1.6
Others: 0.4

Objectives

1. To establish a complete DCT budget management system by the automation of contract data input via RCB, NCI and direct operations data input via OFM and the NCI Budget Office.

2. To develop simulation models of the preclinical drug development process for the purpose of assisting R&D management in the study of expected yields transit times, and costs per compound.

3. Coordinate data pertaining to computer oriented expenditures within the Division of Cancer Treatment.

Methods Employed

1. Through the use of DMB, DCRT program generation software, a single file containing all DCT contracts (commitments, Schedules A&B) and direct operations data is updated monthly at the 2741 terminal. This update is via the DMB, DCRT UPDATE and EDIT system; the data is entered into the WYLBUR Reformat Generator (WYLGEN) for conversion to the appropriate transaction format. This update is designed for the use of nonprogrammers. All entries in the file are classified with respect to stage of DCT program logic (the "old" Linear Array). the "new DCT Program Logic Stratification, the basic priority stratification of the DCT program (Linear Array, Supplementary Array, Exploratory Array), and National Cancer Program objective (Approach 6).

2. Simulation models of the preclinical drug development process for synthetic compounds, natural products (plant and animal material), and fermentation products have been developed as part of an effort to establish quantitative models of the Linear Array in order to enhance program analysis and planning in DCT with the aim of aiding decision-making and allowing for optimum allocation of resources. A FORTRAN program has been written to generate random numbers of arbitrary probability distribution for the simulation of transit times.

3. With the cooperation of the Administrative Officers of DCT, data pertaining to DCRT accounts for DCT is now coordinated by this office.

Major Findings/Accomplishments

1. Programs have been written by DMB, DCRT to generate reports by various combinations of the aforementioned classifications applied to contract and direct operations data as well as by DCT organization. In particular, several new reports are now available for classification of the DCT budget by the "new" DCT Program Logic Stratification. Separate reports have been developed for direct operations and personnel services. Many of the data elements now keyed in via WYLGEN are also captured by the NCI Research Contracts Branch (RCB) Contracts Management System (CMS) on a monthly basis utilizing the same data sources as well as the IMPAC contracts file. The establishment of a complete DCT budget management system requires the automation of contract data input via RCB and direct operations data input via OFM and the NCI Budget Office.

2. Due to the complexity of the preclinical drug development process, a closed-form mathematical representation of the system was not considered feasible. Also, due to the stochastic nature of the system elements and their relationships, empirical methods were not applied. Historical data were available as a series of discrete events, best approximated by arbitrary probability distributions. It was decided to concentrate initially on the distributions of transit time for each compound group. Whenever possible, time estimates were obtained for each step in the drug development process in terms of ranges and peak values. These estimates were then utilized in the form of discrete arbitrary probability distributions. During a run of the computer program, the specified time distributions are generated. From these random values and the designated delay times between steps of the process, a total time distribution is generated. As explorative models, the flow diagrams developed as part of this simulation study have led to a better understanding of the preclinical drug development process and to suggestions for improvement. A matter of particular interest has been the QNS (Quantity Not Sufficient) time estimate for each model. The delay due to the need for the accumulation of sufficient material for further testing is a key step in the process. Correlations between this delay and other time estimates appear to be worthy of further examination.

3. The availability of contract and DCRT account data have enabled this office to formulate the annual ADP plan requested by the OD, NCI. Statistics pertaining to the Data Processing expenditures of DCT are now available via the "new" DCT Program Logic Stratification applied to the DCT budget.

Significance to the Program of the Institute

The computer system that has been developed with the assistance of DMB, DCRT is utilized to monitor and assess budget information related to segments of the DCT program and to more specific project areas both in the intramural areas and in the contract program. The reports generated by this system have led to a detailed description of the DCT research program. Simulation models of the preclinical drug development process provide a means of achieving optimal balance among the various program elements and of allocating the necessary supporting resources according to the volume of work projected.

Proposed Course

TRW, under the direction of Mr. Gerald Brock, has agreed to write the necessary computer programs for the selection and extraction of pertinent DCT contract data from the RCB/CMS data base. The operation of any subsystem developed by TRW will be controlled by RCB; i.e., when a monthly update of the CMS data base is reported by RCB, a DCT transaction file will also be generated. DMB, DCRT, under the direction of Mr. Emmett Ward, will then develop a new update program and appropriate report programs to accomodate the reformatted and expanded DCT transaction file. When a runoff tape of OFM's data for DCT is made available via the NCI Budget Office; DMB, DCRT and DCT will discuss the writing of programs to access this data for use via the DCT transaction file. Also, other programs will be needed to generate DCT direct operations reports now being generated manually by the Administrative Officers.

Consultation arrangements have been initiated with Mr. Edmund Pyle of Smith Kline & French Laboratories to assist in the development and application of simulation models for the preclinical drug development process. In papers that appeared in the Proceedings of the recent Winter Simulation Conference and the August, 1973 edition of *Management Science*, he has developed two simulation methodologies that appear to be applicable to a study of the preclinical development of anticancer drugs. The use of Monte Carlo techniques to simulate the random arrival times at various steps in the drug development process is of special interest. Of equal interest is the development of an utility function that can be used to simulate the preclinical drug development process on the basis of screening success ratios. With the cooperation of DR&D staff, considerable future effort will be devoted to the procurement and study of available data relevant to preclinical drug development.

Automation of the retrieval of DCRT account data from DCRT tapes in the form required by the annual ADP Plans will be investigated. This data combined with DCT Program Logic stratification data will be used to generate reports of DCT data processing expenditures

Honors and Awards

None

Publications

Gordon, M.L.: A Simulation Study of the Preclinical Development of Anticancer Drugs. *Cancer Chemotherapy Reports, Part 3*. (Accepted for publication.)

Serial No. NCI-6402
1. Office of the Deputy
   Director
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 Through June 30, 1974

Project Title: Tumor Growth Kinetics and Chemotherapy

Previous Serial Number: NCI-6109

Principle Investigator: Stanley E. Shackney, M.D.

Other Investigators: None

Cooperating Units: Dr. Marc Straus, VA Hospital, Washington, D.C.

Man Years:

Total: 2
Professional: 1
Others: 1

Objectives:

1. To develop a model which will describe the growth of tumors in such a way that response to various chemotherapeutic agents can be simulated.

2. To apply the growth model to specific tumor systems.

3. To explore the pharmacodynamics of chemotherapeutic agents in specific tumor systems with the aid of the model.

4. To develop an approach to chemotherapy of tumors based on the expected interaction between tumor growth kinetics and drug pharmacodynamics.

Methods Employed:

Computer modelling techniques, *in vivo* and *in vitro* cell propagation techniques, radioautography with tritiated thymidine.

Major Findings:

1. Modelling Studies

a. Over the past year multiple kinetic parameters have been explored with the aid of the model, including multithreshold PLM curve studies, the labelling index, grain count halving methods, long term infusion studies, DNA content distributions, and combined DNA content-tritiated thymidine studies. Major findings include the following:

i. All tritiated thymidine associated parameters are dependent on relative radioautographic exposure and counting threshold: In general, then, absolute values for kinetic parameters obtained at single thresholds offer little useful kinetic information in heterogeneous populations. Specific biological kinetic information can be obtained from:

ia. The relation between the labelling index and labelling intensity of labelled cells,

ib. The patterns of mitotic labelling at multiple grain count thresholds in the PLM curve,

ic. The relation between the mitotic index and the labelling index at low thresholds as a function of time in long term infusions.

id. The patterns of labelling intensity as a function of time by DNA content class.

b. During the past year, two human cancers have been examined in detail with the aid of the model. Multiple kinetic parameters in adult acute leukemia and malignant melanoma were simulated. The simulation studies in melanoma will be completed within the next month or so. Major findings include the following:

i. When PLM curve simulations correspond with the observed data, other kinetic parameters fall neatly into place. These include the labelling index, grain count halving, long term infusion studies, and presumptive growth curves. This provides strong validating support for the model.

ii. Significant kinetic differences between adult leukemia and melanoma were demonstrated.

iia. Mean and median cell cycle times are considerable longer in melanoma than in leukemia.

iib. The variance in cell cycle times is less in melanoma than in leukemia. That is, there is a smaller fraction of rapidly proliferating cells in melanoma, and

iic. The rate of DNA synthesis in the slowly proliferating cells in melanoma is low and is spread out over a long period. In leukemia there is a relatively sharp peak of DNA synthesis toward the end of the cycle, even in very slowly proliferating cells.

This is the first demonstration of meaningful quantitative kinetic differences in human populations which correlate with their differential responsiveness to chemotherapy.

c. The in vitro simulation package has been completed and is operational. Preliminary simulation studies of our S180 system in vitro were begun this year.

d. The drug simulation package has been written, but has not yet been tested and debugged. Extensive work on drug simulation in *in vitro* systems will be carried out in the coming year, when the simulation studies on human tumors are completed, and when the in vitro are drug studies completed (vide infra).

e. An evaluation of the Gompertz equation in Ca755 was carried out in collaboration with Dr. Marc Straus. Serious defects in the Gompertz equation were found. This work is being prepared for publication.

2. Experimental Studies

a. PLM curve studies

In the past year multithreshold PLM curve and labelling index studies in late growth *in vitro* have been completed. DNA content distributions were obtained at various stages of growth, and were found to correlate well with the PLM curve studies. A paper describing this work will appear in *Cancer Research* within the next several months.

b. Bone marrow studies

A study of kinetic-microarchitectural correlations in the unperturbed mouse marrow has been completed. A paper describing this work has been prepared for publication. Major findings include the following:

i. There is a considerable degree of kinetic microarchitectural organization in the marrow. Rapidly proliferating cells are found in the subendosteal region, megakarocytes are found peripherally, and mature granulocytes are found more centrally, often lining the sinusoids.

ii. In the steady state it would appear that there is a central migration of cells, with presumptive removal via the sinusoidal system.

iii. These studies suggest a different model for marrow stem cell kinetics than the one that is currently accepted.

c. Drug studies

During the past year cell cloning methods were developed for our S180 *in vitro* system, and studies on the effects of hydroxyurea and ara-C were begun.

The objectives of these studies are two-fold:

i. In view of the demonstration in our system that DNA synthesis occurs throughout much of the cycle, but at low rates during "G_1" and "G_2", it was felt important to explore the effects of "S phase" specific agents on "non S" cells, to see if the postulated multiple sites of action of such drugs can be subsumed under a more unifying principle, i.e., DNA synthesis rate dependence.

ii. We believe that stage specificity (or DNA synthesis rate dependence) are not adequate to describe the kinetics of drug interaction. Such relations as degree of sublethal damage per unit dose and recovery rate per unit damage are important determinants for multiple dose and combination scheduling, and may differ from one "S phase" drug to another.

A preliminary analysis of our cloning data suggests that hydroxyurea differs markedly from ara-C in its dose response, degree of continued cell kill with time, and rate of recovery from sublethal damage. These conclusions will require confirmation by repetition of the experiments, and the use of radioautographic and DNA content distribution measurements.

d. In collaboration with Dr. Straus, the kinetic studies we have done *in vitro* are being performed in S180 *in vivo*. The dependence of the PLM curve on threshold and relative exposure has been confirmed, and the changes in the DNA content distribution with population aging are comparable to those seen *in vitro*.

Significance to Biomedical Research and the Program of the Institute

Hopefully, a better understanding of the growth characteristics of tumors, and the way in which growth affects response to chemotherapeutic agents, will lead to a rational, systematic approach to the chemotherapy of tumors, both experimentally and clinically, and provide the means for translating experimental data obtained in animals into clinically useful information.

Proposed course

1. Modelling studies

Modelling studies are expected to continue on several fronts:

a. The simulation of human tumor kinetic data as they become available,

b. The simulation of *in vitro* kinetics in our S180 systems,

c. The simulation of drug effects in our *in vitro* system,

d. The simulation of S180 kinetics *in vivo*.

e. The prediction of *in vivo* drug-population interactions in S180, and

f. Preliminary simulations of human tumor-drug interactions *in vivo*.

2. Experimental studies

a. PLM curve studies
The collaboration with Dr. Straus in collecting kinetic data in S180 *in vivo* is expected to continue.

b. Bone marrow studies
Studies on kinetic-microarchitectural changes in the marrow following perturbation by ara-C are planned for the coming year.

c. Drug studies
In vitro drug studies comparing hydroxyurea and ara-C will continue. Cloning methods, radioautography DNA content distributions, and total cell counts will be used. Parallel studies in S180 *in vivo* are planned in collaboration with Dr. Straus.

d. Cell Sorting

A four parameter cell sorter is being constructed for us by the Los Alamos Scientific Laboratory, to be delivered in mid-to late 1974. Combined DNA content distribution-labelling studies are planned.

3. Clinical studies

a. Dr. DeVita has expressed a stong interest in obtaining needle aspirates of lymphomatous lymph nodes for purposes of quantitative histopathologic classification and the monitoring of kinetic perturbation by drugs, using the cell sorter. In the coming months it is anticipated that the methodology of specimen preparation will be worked out in collaboration with the Los Alamos Scientific Laboratory.

b. A collaborative effort with Dr. Straus is being considered for tritiated thymidine long term infusion studies in animals and man.

Publications:

Shackney, S.E., A Cytokinetic Model for Heterogeneous Mammalian Cell Populations. II. Tritiated Thymidine Studies. The Percent Labelled Mitosis (PLM) Curve. J. Theoret. Biol. 44: 49-90, 1974.

Shackney, S.E., Ford, S.S., & Witting, A.B., The Effects of Counting Threshold and Emulsion Exposure Duration on the Percent Labelled Mitosis Curve and Their Implications for Cell Cycle Analysis. Cancer Res. 33: 2726-2731, 1973.

Serial No. NCI-7025
1. Office of the Deputy
   Director
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 Through June 30, 1974

Project Title: Biomedical Engineering for Intramural Research, DCT

Previous Serial Number: None

Principal Investigator: William Z. Penland

Other Investigators: NCI: Seymour Perry, Paul Carbone, Michael Walker, Vincent Oliverio, Anthony Kontaratos, Kwang Woo, Stanley Shackney, Lewis Lipkin, Arthur Levine, Robert Graw, Joan Bull, Peter Lohrman, Jeoffrey Herzig, Philip Waalkes, Philip Schein, Marc Straus, Cephas Patch, Vincent DeVita, Oleg Selawry, John Ziegler, Prem Sarin, John Wood, Frank Quinn, David Johns, Robert Gallo, Vincent Bono
ADL: Alfred Wechsler, Leonardo Peusner, Phillip Thayer, Alan Burg, Edward Cook, Erica Drazen, Donald Lindsay, Robert Liss, Karl Wiig, Bruce Shapiro, Jack Ennis, Peter Kaiser, Lita Nelsen
RTI/NASA: Thomas Wooton, Fred Spross, Thomas Williams, Robert Zimmerman
ORNL: Julian Breillatt, Norman Anderson
ABCOR: Lee Nuwayser, Richard Anderson, James Porter
Los Alamos Scientific Laboratories: Paul Mullaney, Harold Crissman, Gary Salzman
Bionetics: David Gillespie, Carl Saxinger
NIH Clinical Center: Gerald Johnston, Michael Green

Cooperating Units: Division of Cancer Treatment Program Staff; Office of the Deputy Director for Program, DCT; Medical Oncology, DCT; Experimental Therapeutics, DCT; Drug Research and Development, DCT; Baltimore Cancer Research Center, DCT; Nuclear Medicine Department, Clinical Center, NIH; Research Triangle Institute; Oak Ridge National Laboratories (ORNL); Arthur D. Little, Inc. (ADL); Sci-Med, Inc.; Abcor, Inc.; NASA, Goddard Space Flight Center; Litton Bionetics; Los Alamos Scientific Laboratories (LASL); Entropy Limited; Carnegie Mellon University

Man Years:

Total: 15.5
Professional: 14.5
Others: 1

General Objectives/Scope

1. To provide specialized and broadly based biomedical engineering (BME) resources for the Division of Cancer Treatment, NCI.

2. To provide definition and implementation of BME projects responsive to changing priorities of diverse research programs, projects and tasks of individual investigators of the Division.

3. To test and refine approaches for effective application and management of broad BME resources through the contract mechanism.

Major Findings/Accomplishments

In direct support of new and continuing clinical and pre-clinical investigations of the DCT Program, a broader range of BME activities were accomplished this period. Specific instrumentation developments as well as exploratory engineering studies were expanded. DCT investigators have found that substantial accomplishments have arisen from interaction with engineers competant to undertake diverse BME studies and developments.

Instrumentation-Cellular DNA Studies

Considerable engineering assistance was directed to obtaining a new flow-system instrument for quantitative analysis and sorting of biological cells, based upon multiple measurements of physical and biochemical properties. An agreement with the Los Alamos Scientific Laboratory, AEC, will accomplish R&D with instrumental and biological techniques to progressively adapt a prototype cell microfluorometer and computer data analysis system for DCT investigations. This instrumentation is expected to be used initially for human tumor cell characterization and drug evaluation studies (Shackney, Bono, et al.). The instrument is capable of individual cell sorting at rapid rates (50,000 cells/minute) as determined by multiple cell parameters of size and fluorescence. Measurement of these parameters at rapid rates indicates many potential experimental and clinical uses for obtaining precise DNA content distribution of cell populations as well as for immunological cell sorting studies. The rapid accumulation of data of DNA synthesis patterns of the cell cycle both in the normal state and following drug administration promises to (a) elucidate cycle stage dependence of drugs; (b) assist initial kinetic tumor classification (c) speed up the acquisition of cell kinetic information needed in several intramural investigations; (d) permit clinical monitoring of various drugs in tumor and normal tissues, and (e) permit new studies of drug effects in *in vitro* and *in vivo* cell populations.

ADL has developed and demonstrated a microtechnique facility for improving the kinetics of hybridization of cellular DNA to rival RNA. Hybridization experiments (Gillespie, Saxinger) required a facility to accurately measure, store, and separate solutions of 0.1λ (10^{-7} liters) or less. This was achieved by developing an instrument consisting of a micropipette reaction vessel with a microinjection system. The present system can consistently carry out reactions in volumes as low as 0.04λ (± 10%). This represents a twenty-five fold improvement over existing state-of-the-art techniques.

Protective Environment Studies

Engineering support of the protective environment study (Levine, et al.) was furnished through several ADL projects and consultation. The evaluation of resources needed to support laminar air-flow rooms was completed this period. Intramural reports by ADL and published results (Drazen, Levine, *Hospitals, J.A.H.A.*, Vol. 48, 88-93) indicate the impact of these rooms upon operations, costs, staffing levels and support services of research institutions supported by DCT programs. The NCI staff has effectively used this information, with assistance from ADL, to address numerous inquiries from the U.S., Japan, U.S.S.R. and other countries.

Clinical evaluation of a unique prototype bacteriological isolation garment during this period has found the basic design satisfactory and indicates the garment concept should be useful to the care of patients requiring treatment in protective environments. Drawings and specifications of the garment will be made widely available to interested manufacturers, research institutions and individuals. Publication of the garments' development and clinical assessment is anticipated (The Lancet).

An NCI/ADL project is being planned to adapt present LAF rooms to accomodate specific requirements of pediatric care anticipated in solid tumor programs. Prospective studies include; the investigation of microbial protective measures that could increase parent/child contact, the design or aquisition of new interior furnishings, the support of unique pediatric nursing requirements, etc.

Engineering planning assistance was given to visiting scientists, Institute of Experimental and Clinical Oncology, U.S.S.R., and to visiting Japanese engineering consultants regarding the establishment of large LAF facilities in Moscow and Tokyo.

Drug Activity Analysis

A trial effort will utilize a pattern discovery program to extract physical/chemical features of drug compounds which correlate to anti-tumor activity and toxicology. The project will be a joint effort by DRD Branch (Quinn, Wood, et al.), Entropy Limited, the Carnegie Mellon University and ADL. Initial research efforts will attempt to: (1) Find patterns in structural variations that correlate with compound activity in samples of approximately 100-200 compounds of similar structure which have been tested for L1210 tumor activity, where the sample includes both active and inactive compounds. (2) Find patterns in structural and physical information which correlate with varying degrees of activity in samples of 40-50 pre-clinically active compounds representing a wide range of dissimilar structures and known activities.

Leukocyte Procurement

Two conceptually different rotor designs for the efficient collection of human granulocytes by existing continuous flow blood cell centrifuges, are undergoing early feasibility tests and modifications at The Oak Ridge National Laboratories. A newly completed particle-fluid mechanics model is being used extensively to modify design faults and to address design and operating criteria of both rotor systems as well as the newly developed automatic control system for stablizing the buffy coat/RBC interface through hematocrit monitoring. This control system has shown promise in early tests using a conventional rotor and will be vital to optimum granulocyte collection by the prototype radial-flow rotor. First collections using the prototype axial-flow rotor and a variety of bloods have been disappointing due to critical flow factors associated with the red cell phase and the 4° slope of the separator channel. Before design corrections were made, this system did collect 24% of the input granulocytes (dog) with 7% RBC by volume under abnormally slow feed rates.

Transmission and scanning electron microscopic methods have been used by ADL to define the processes of granulocyte adherence and elution from nylon fibers (Leukopak). Fiber collected granulocytes have a decreased viability and are morphologically compromised. Isolation of granulocytes with minimal cytopathology presumably will not only increase the half life of these cells in vivo, but also will make available WBCs with an essentially intact cytologic competence. ADL data has provided an understanding of the interaction of white blood cells with the nylon fiber column and has led to an hypotheses for storing granulocytes as cells adherent to the Leukopak fibers. Such data may provide a basis for designing new or modifying existing filter systems in order to maximize the yield of viable, competent granulocytes.

Testing of the ABCOR centrifugal elutriator rotor at the NCI is continuing following the successful correction of drive system problems involving dynamic balancing and speed range. While early mechanical problems have been systematically eliminated in tests with dogs, the IBM seal has been found inadequate for the rotor to spin at 2000 rpm. Several relatively simple corrective measures are being tried to prevent further breakdown of the fluid saline seals in order to gain adequate trials for this rotor system.

Mathematical Cell Kinetics Model

A cell kinetic model (Woo, Wiig) has been further developed this period to simulate the growth behavior of tumors under drug treatment and to simulate the distribution (and synthesis) pattern of polyamines in the spleen of mice as a biomarker for L1210 leukemic cells. Simulation results indicate that the model is well suited to study cycle-phase specific drug effects, and also that it is required to distinguish between drug effects on mitotic inhibition, increased cell losses or changes in the fraction of mitotic daughter cells that remain proliferating. The model has been used to predict the surviving fraction in experiments where the surviving fraction was established by bioassays.

The model enables one to predict and analyze various properties of the cell cycle, including the number of cells in each phase at any point in time, the actual drug effects, the specific growth rate, and the growth fraction, etc. It also provides a framework to predict possible behaviors of the tumor under synergistic effects of two or more drugs.

The immediate aim for further development of the model is to emphasize the clinical implementation in collaboration with clinical investigators. Accordingly, attempts are presently under way to develop and investigate a basis for

Solid Tumor Imaging

This period, through collaboration of the ADL staff and members of the Nuclear Medicine Branch, C.C. and of the Medical Oncology Branch, DCT, a systematic survey of advanced developments in x-ray and radioisotope instrumentation is being made for potential diagnostic support of Medical Oncology programs. Technical seminars are being planned for review of acoutical holography techniques, radiographic imaging with electron radiography techniques, multi-wire proportional counting chambers and other current developments from a variety of sources.

Miscellaneous BME Projects

An ADL project was initiated to develop an inexpensive solid-phase TINE-Type disposable skin-testing device and assay system for large-scale testing of delayed hypersensitivity in man. A useful device would apply up to six antigens uniformly to the skin and present the best physical arrangement of tines to obtain confluent introduction. In a pre-design survey, ADL identified a partially developed product (Lincoln Labs) and source of antigens capable of meeting NCI requirements much sooner than anticipated. The NCI is in the process of negotiating a contract with Lincoln Laboratories, Peoria, Illinois.

Transmission electron microscopic studies by ADL have generated data on the expression of virus particles in IUdR-treated cultures of AKR cells (Levine, et al.). An unanticipated but significant finding was the observation of virus-like particles in several control as well as IUdR-treaded AKR cells. This ADL study is continuing. A cooperative study involving the heteroduplex mapping of viral DNA and T-antigens (Patch, Levine) will be assisted by ADL microscopy resources.

A new ADL project will develop and demonstrate a miniature profusion device for laboratory animal studies designed to monitor the effectiveness of chemotherapeutic agents in directly profused organs. Large commerically available systems are not readily adaptable to small scale use since blood priming volumes are disproportionately large for rodents. The miniature device will be designed for either isolated organs or animals while disconnected from the main circulatory system and will permit easy access of drugs and removal of fluid samples.

In support of radioautographic measurement of concentration of ^{3}H-labelled DNA in mammalian cells (Shackney), ADL investigated the feasibility of a novel method for enhancing the efficiency of radioautographic detection of ^{3}H beta radiation from moderately thick samples. The method consisted of homogeneous infusion of a dilute solution of a conventional primary scintillator such as 2.5-diphenyl-oxazode ("PPO") in xylene into cellular material on fixed tissue sections mounted on microscope slides, followed by contact-exposure of the treated specimen to light-sensitive photographic plate or film, which is subsequently developed, fixed, dried and replaced over the tissue section for microscopic examination. Experimental work proved the feasibility of the infusion method as a modification of the conventional tissue deparaffination and fixing process, but the specific technique employed was found to suffer from several deficiencies which made it inferior to the conventional method.

application of the cell kinetic model to clinical and experimental situations, which support quantitative studies of drug actions in relation to phase specificity in particular tumor systems, and also of determining the degree of inhibition in S-phase, mitotic loss and the number of mitotic daughter cells that become nonproliferating. Investigation into the applicability and mechanisms of biochemical markers may be included. Specific ADL projects will continue to support this collaborative modeling activity.

Automatic Image Processing

Substantial engineering support for the DCT investigation of automatic blood cell identification studies (Lipkin, et al.) was provided by two ADL projects. Two phases of one project have been completed towards evaluating the most desirable artifical intelligence 'question/answering system' and related software to be used with the current automated microscope/computer system. Continued activity is expected in this project to assist the design and development of hardware interfaces and components for implementation of the 'question/answering system' selected. The development of compiler for the automated scanning microscope by ADL is nearling completion. This work consisted of creating descriptive software documents (general picture processor), designing a language for the compiler to relieve the programmer of tedious and error-prone procedures, and developing an operational compiler with ancillary software programs.

Initial project proposals by ADL are being considered to investigate high intensity arcs, tunable dye lasers, as well as optical approaches for improving the luminous intensity of state-of-the-art light sources for advanced image processing systems.

Collaborative NASA Developments

NASA technology transfers and engineering assistance (Wooten, RTI) during this period have involved the application of instrumentation for DCT cryopreservation studies (Bull, Graw) as well as an advanced imaging system for solid tumors (Johnston, DeVita). A unique freeze/thaw apparatus, conceived at the Jet Propulsion Laboratories and reduced to practice at Goodard Space Flight Center, has been delivered and successfully demonstrated to DCT investigators. This device offers considerably more precise and responsive control of freezing rates for experimental preservation studies of human leukocytes than available NCI systems or commercial devices. A novel multi-wire proportional counting chamber (NASA/Ball Brothers Research, Inc.) proposed for detecting the position of ionizing particles with low spatial resolution has been extensively reviewed this period (DCBD, Green, Penland, et al.) along with competing solid germanium liquid xenon developments (UCLA, ORNL, Lawrence Radiation Labs). The proposal was rejected in favor of a competative camera development if warranted by future DCT solid tumor studies.

A presentation of the DCT Biological Isolation Garment development was made before The House Subcommittee on Science and Technology, March 5, 1974 (Penland) upon request from NASA's Technology Utilization Program.

An ADL project will formulate plans for a computer system to assist recording, storing, manipulating, and displaying data of biopsy slides used extensively in cell kinetic and drug therapy studies on patients with lung cancers (Straus).

ADL engineering and architectural assistance was given to the Baltimore Cancer Research Center (BCRC) towards developing a relocation plan involving new facilities of the University of Maryland Hospital. Initial information gathering, the preparation of detailed checklists for critical items, conceptual layouts and early logistical planning was accomplished with the BCRC staff.

Major Consultations

DCT investigators were provided consultation on the following subjects principally by ADL specialists: Filtration of IV Fluids and Medications (Gallelli, DeVita); Demonstration of a Novel Manipulatable Bronchoscope Device (Cohen, Selawry); Feasibility of Improving Luminous Intensity of Light Sources for Image Processing Systems (Lipkin); Assistance in Providing Information on Laminar Flow Facility Operations (Levine); Use of Indwelling Alloy Needles to Prevent Clotting (DeVita); Assistance in Testing Optical Filter Device (Lipkin); Use of ^{3}H-Bleomycin in Radiation Therapy (Perry); Assistance in Examination of Use of Cell Microfluorimeter/Cell Sorter (Penland/Shackney); Review of Differential Counting Instrumentation (Herzig); Dynascope Demonstration and Its Applications (DCT investigators); Survey of Cell Culture Techniques (Waalkes); Examination of Field Ionization Mass Spectrometry for Biomarker Assay (Waalkes); Review of Microchannel Plate Amplifier for Grain Counting in Tissues (Penland); Assistance in Specifying Acousto-Optic Filter for Purchase (Lipkin); Examination of Centrifuge Flow Patterns (Penland); Provision of Glass and Nylon Beads for Filtration Leukapheresis (Lohrman).

Significance

The Division of Cancer Treatment has a large number of problems that can potentially benefit from bioengineering approaches. Recent expansion of BME activities has been possible through the NCI-ADL contract. This contract represents a novel kind of interaction and mechanism which has been, on the whole, successful in gaining acceptance and confidence of NCI investigators. The achievements of the projects constitute patient, incremental but very useful additions to the knowledge and capabilities of the investigators involved. The projects have immediate impact on the ability of the investigators to achieve specific goals, and may also in some cases have a longer range, broader impact.

Proposed Course

Emphasis will be given to identifying key technical problems of research programs that are amenable to BME approaches and to obtaining technical assistance outside of present contract resources on particularly complex problems. Future activities will continue investigations and projects in the research areas addressed in this report. Unexplored areas to be examined include: Rapid Measurement of DNA synthesis rates to study effects of anticarcinogenic agents, improvement of DNA-RNA hybridization kinetics for studying viral genomes, improvement in fluorescent cell sorting procedures and instrumentation, improvement of analytical procedures and instrumentation for screening of specific biomarkers, application of quantitative techniques for tumor cell number and biomarker assays involving newer tissue culture growth chambers, design of hypothermic infusion techniques and instrumentation, the application of multichannel plate amplification devices to tissue culture autoradiography, the application of field ionization mass spectrometry to clinical drug distribution studies of solid tumor materials, the application of commercial drug delivery systems involving implantatable and intravenous devices, and the application of magnetic imaging techniques for lung tumor diagnois.

Publications

Technical reports are compiled on all appropriate biomedical engineering projects primarily for the use of the intramural staff and are furnished the National Technical Information Service, U.S. Department of Commerce. Publications, honors and awards, lectures, etc. resulting from these efforts generally are reported under the individual research projects, and are obtainable from the Biomedical Engineering Unit, DCT.

Serial No. NCI-7070
1. Office of the Deputy Director
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 Through June 30, 1974

Project Title: Automated Grain Counting/Densitometer

Previous Serial Number: Same

Principal Investigators: Lewis E. Lipkin
Peter F. Lemkin

Other Investigators: Marta Jo Wade
Morton Schultz
Bernice S. Lipkin
George Carman
James Bryan
Sprague Hazard

Cooperating Units: Computer Center Branch, DCRT
Biomedical Engineering and Instrumentation Branch, DRS
Carman Electronics, Corvallis, Oregon

Man Years:

Total:	5.6
Professional:	2.6
Others:	3.0

Project Description:

The objective was to construct an instrument which would allow a user (technician or physician) to classify cells, particularly blood and/or marrow cells, in radioautographs and have automatic nuclear and background grain counts performed for each cell so classified. The project involved the interfacing of some commercially available quantitative television microscopy equipment, a general purpose computer and its peripherals, and some specially designed illumination, microscope and general optical equipment. In addition to hardware construction, something in the neighborhood of 25,000 lines of programming was necessary to complte the facility for a habitable human use of the equipment.

Field Model Grain Counter Construction and Maintenance: The Grain Counter was functionally complete at the beginning of the fiscal year. The heretofar implicit responsibility of the Image Processing Unit for supervision of construction and maintenance of several field models now becomes explicit and impelling. With a device as complex as the Grain Counter, it seemed increasingly unlikely that (despite the seeming efficiency and economic desirability of such an approach) we would be able to completely delegate the functions relevant to field model redesign, optimization, repackaging, debugging and maintenance.

Nevertheless, because of our special relationship with A. D. Little, their acknowledged wide range of relevant expertise, and in the hope of saving several months in the procurement process, we devoted a major effort during the first half of the reporting period to exploring the possibilities of a sole source procurement. These expectations foundered largely because of the relatively large expense. Essentially, the fabrication of one or more units would entail the expense of the equivalent of an almost complete redesign and the associated learning process (6-12 months) by several outside engineers.

This completed the lesson. In designing a system for external as well as in-house use, there is no alternative (at least in our milieu) to continued participation by the original design staff in the field model phase. Once again, we were forced to defer work on the automatic cell analysis design system. First priority is assigned the task of putting field models of the Grain Counter into the hands of those who could and would evaluate the device. Further, the development and responsibility of the original team in field maintenance and debugging, as well as in the substantive field trials, is both necessary and desirable and is therefore planned for.

In order to make maximal use of our particular knowledge and experience while deviating our efforts minimally from other equally (long term) important tasks, we devised a multicontract approach for the field models. (The formulation and execution were greatly aided by the staff of Negotiated Contracts Unit, Office of Assistant Director for Materiel Management.) Briefly, by dividing the field model procurement process into four major tasks, and undertaking the preprocurement managing process, we have greatly increased the chances for reasonable cost coupled with increased certainty of acceptable performance.

Contract I was a professional services contract with Mr. James Bryan (formerly Chief, Section on Technical Development, NIMH). He undertook the preparation of the formal specifications, both for sources sought and final requests, for proposal actions. This permitted us a channel wherein our technically detailed but informal specifications could be effectively formalized. His other duties will include liaison during the vendor selection process and later with the successful vendor.

Contract II with Carman Electronics calls for the redesign of the interface. The staff of Carman Electronics includes the original designer of the interface, a particularly cost effective measure in this context. In addition, following return of the first set of cards which constitute the interface, the designer will debug these units providing us in the process with what will be the basis for a set of maintenance programs.

Contract III with Cambion Thermoelectric is for the fabrication of custom made circuit cards and their automatic wire wrapping according to specifications developed under Contract II. We expect to use this contract, amended if necessary, to provide all custom electronics (except for one Digital Equipment type card) for all of the field model Grain Counters.

Contract IV now in procurement process will be for the assembling of component parts, installation and field maintenance of the Grain Counter. The vendor will purchase all off-the-shelf components, while the government will provide all unique components such as the requisite circuit cards. Thus, those items which are most unique and/or specialized remain under the direction and control of the original design team.

An ad hoc review group for final evaluation of the response to the request for proposals has been formed. This includes engineering personnel from NCI, DCRT, NINDS and NIMH.

The response to the sources sought solicitation was extremely encouraging in that the eight respondents, though of varying size, were all well qualified in this highly specialized area. This allowed much tighter specifications to be written for the final contract proposal.

The Grain Counter presently in operation within the Image Processing Unit will be brought into electrical identity with the field models.

At the end of the reporting period, human derived autoradiograms were being collected for a first round-robin sequence of field tests in which the same material (independent of cell classification) will be counted by the four field and the NCI instruments.

Technical Progress: For the most part, work on this aspect of the Grain Counter consists of attention to large numbers of minutely detailed points and problems. Solutions to individual such items may seem of minuscule importance, yet taken together they were necessary for the conversion of the Grain Counter from something that works to an instrument that works well.

Redesign of the interface has been a group activity with primary responsibility vested in Carman Electronics. The design has reduced the physical complexity of the interface with consequent favorable effects on future debugging and maintenance functions. A measure of this is given by the replacement of nearly 100 Digital Equipment and custom circuit cards of varying size by nine standard large sized Cambion cards and one card for direct insertion into the PDP-8 Omnibus.

During the first quarter, the design for the solenoid driven Zeiss filters by Sprague Hazard was completed, thus liberating the Grain Counter microscope completely from the original optical bench design bed.

Schultz developed a procedure for effective optical density filter matching which allows an increase in speed of operation. He has also gone into the Quantimet subsystem in considerable depth, devising modifications for circuits such as the automatic gain control which will also allow increased speed of operation. He has worked closely with the local and national optical groups at Carl Zeiss in optimizing the illuminating system and the microscope-Plumbicon camera interface. The properties of the video signal derived from the Plumbicon have been deeply and extensively examined by Schultz with significant improvements in prospects for maintenance.

The classification algorithm has been extensively refined, debugged and expanded by Peter Lemkin over the course of the year. He has added some software "prologs" so that the oridinary user may restructure the Grain Counter functions at the time of initial sign-on.

Lemkin has also been responsible for a series of programs for the Grain Counter system which have allowed for:

a) Evaluation of the densitometer function via an extensive analysis of variance.

b) Determination of some size, shape and densitometric properties of varying aspects of the L-1210 leukemic cell cycle.

c) An autofocus routine which could be incorporated into the Grain Counter operations proper if needed by a particular user.

d) Programs to aid in development of new morphologic descriptors, e.g. texture in relation to white cell maturation.

e) Analysis of materials generated by Duke University group under contract for improvement of autoradiography.

f) Feasibility testing of double label counting.

g) Real time tracking of particulate matter (mitochondria) in living isolated neuronal axons, as seen under Nomarski interference contrast.

Substantive Work Employing the Grain Counter:

a) M. Wade and B. Lipkin are conducting a detailed analysis of the size, shape,densitometry and textural parameters associated with successive phases of the L-1210 leukemia cell cycle.

b) Grain counting per se on various random autoradiographs during the first three quarters was devoted to stressing the system in order to expose the more obscure bugs present in any system of such complexity.

c) A study with B. Lipkin on the textural properties of association with progressive leukocytic maturation has been completed and accepted for publication.

d) Using the existing Grain Counter, we have explored its use for tracking low intensity Nomarski images of particulate material in the axons of living neurons. Signal to noise problems made conventional time-lapse cinematography unacceptable. The Grain Counter was able to automatically generate the X-Y coordinates of the moving body and write a tape for subsequent analysis. This study was undertaken as a test of the low light level characteristics of the Grain Counter and in cooperation with Drs. Clifford Christian and Anthony Breuer, NICHD.

e) The Grain Counter is in systematic use by the Duke University group in quantitating their results. The object of this program is the improvement (photographic parameters) of autoradiographic processing.

Honors and Awards: None

Publications:

Lipkin, L.E., Lemkin, P. and Carman, G.: Automated autoradiographic grain counting in human determined context. J Histochem Cytochem. In press.

Lipkin, B.S. and Lipkin, L.E.: Textural parameters related to nuclear maturation in the granulocytic leukocytic series. J Histochem Cytochem. In press.

Serial No. NCI-7071
1. Office of the Deputy Director
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 Through June 30, 1974

Project Title: Automatic Cell Analysis

Previous Serial Number: Same

Principal Investigators: Lewis E. Lipkin
Peter F. Lemkin

Other Investigators: Marta Jo Wade
Morton Schultz
Bernice S. Lipkin
George Carman
Sprague Hazard
Bruce Shapiro
Peter Kaiser

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS
Computer Center Branch, DCRT
Carman Electronics, Corvallis, Oregon
Arthur D. Little, Cambridge, Massachusetts

Man Years:

Total:	4.1
Professional:	3.6
Others:	0.5

Project Description:

The objective is the construction of an advanced "designer;" a general purpose apparatus to allow the on-line investigation of the morphological properties of a class of related biological images such as the cells in a marrow smear. The object of such investigation is to develop an optimal set of properties and morphologic relationships which will allow the automatic classification of members of the classes, their quantitation and characterization.

Because of the required concentration on the autoradiographic Grain Counter and insufficient personnel, work on this project was delayed in both hardware and software. Major design principles were decided and small fragments of the real time picture processing system design reached the wire list level of final design in collaboration with Carman Electronics. Examples of such include the "E bus" which is the major mode of communication between the local process control computer, the 8e and such component subsystems as the Quantimet. The buffer memories are at almost design level since we have evaluated the random access memory integrated circuit to be so employed.

We have completed procurement procedures on the Axiomat microscope and are awaiting delivery at this writing. This choice for the microscope component seems particularly suitable because of its symmetry, the infinity corrected optics, and the consequent ease of direct interfacing of one or more scanners at almost any point in the microscope's optical train.

Procurement procedures for the random access wavelength control device have been completed and here too we are awaiting the arrival of the subsystem. During the year we were able to define and begin procurement on a suitable monitor for this tunable optical filter - a rapid scan spectrometer which should be here during the first quarter of fiscal year 1975, in time for the definitive tests of the device.

The special purpose TV scanning microdensitometer, a special version of the Quantimet 720, has been delivered and is in (stand-alone) operation. The newer modules are being analyzed by our group in order to bring the level of certainty in the interface designs to that attained in the Grain Counter equivalent.

The general purpose computer noted in last year's report has been delivered and is operational in stand-alone mode. It is complete except for one disk drive and installation, of which we are expecting daily at this writing.

Taken together, items 5 and 6 of last year's report, the interface and special purpose hardware gray scale Picture Processor are in reality a special purpose computing device. We term it the GPP. This has the structure of a three-address machine with 4,000 words of program memory and a specially designed instruction set peculiarly suited to the needs of real time picture processing. The design principles of the various bus structures, the instruction set, the buffer memories and its relationship to the controlling PDP8/e have all been established. The design outline has been documented in a more than 100 page document on the Real Time Picture Processor. Additions are being made almost daily at the end of the current year.

The vital role to be played in the overall system by the DCRT PDP-10 has been documented in both the open literature and in much greater detail in our in-house documentation. Peter Lemkin and Bruce Shapiro have been particularly active in this regard. The larger general purpose computer has been upgraded to a dual processor during the reporting year, thus making it even more likely that we will be able to fully utilize its potential power for our project during the next and succeeding years. Schultz has added greatly to this potential by designing and building high speed digital communications hardware for both our PDP8/e and the PDP-11 message switcher. The latter is our link to the remotely located PDP-10. Bruce Shapiro has developed a message switcher monitor for our 11. This demanded particularly close collaboration with the DCRT PDP-11 group in order to ensure compatibility. The smooth and effective interaction was particularly gratifying.

The software areas noted as items 8-11 in last year's report have been pursued. Of particular note is the progress made by Lemkin in development of an analytic model to reside at the top system level. He, along with Shapiro, is attacking the problem of an interpreter to serve user and RTPP requirements for full system operations. These programs, grammars and the high level compiler are being written in SAIL, the Stanford Artificial Intelligence Language, available to us on the PDP-10.

Peter Kaiser is also using SAIL as the vehicle for construction of a compiler for the GPP and for the controlling PDP-8. We expect that completion of this, the associated linking loader, and the debugging package should be operational during fiscal year 1976.

Honors and Awards: None

Publications:

Lemkin, P., Carman, G., Lipkin, L., Shapiro, B., Schultz, M. and Kaiser, P.: A real time picture processor for use in biological cell identification. I - Systems design. J Histochem Cytochem. In press.

Carman, G., Lemkin, P., Lipkin, L., Shapiro, B., Schultz, M. and Kaiser, P.: A real time picture processor for use in biological cell identification. II - Hardware implementation. J Histochem Cytochem. In press.

Shapiro, B., Lemkin, P. and Lipkin, L.: The application of artificial intelligence techniques to biological cell identification. J Histochem Cytochem. In press.

Schultz, M., Lipkin, L., Wade, M., Lemkin, P. and Carman, G.: High resolution shading correction. J Histochem Cytochem. In press.

Serial No. NCI-7085
1. Office of the Deputy Director
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Kinetic (Quantitative) Studies on Tumor Cell and Biomarker Interactions; and on Cell Age, Cell Size, and DNA Content Distributions and Their Implications to Cancer Chemotherapy

Previous Serial Number: same

Principal Investigator: Kwang B. Woo

Other Investigators: G. P. Sarna, Seymour Perry, M. Kim and M. Straus

Cooperating Units: Department of Electrical Engineering, Cornell University; and VA Hospital, Washington, D. C.

Man Years:

Total: 1.7
Professional: 1.4
Other: 0.3

Project Description:

Objectives

1. To establish a quantitative relationship between tumor cells and their specific biomarkers. A mathematical model is developed which by using the concentration measured of a biomarker and the characteristics by its kinetic parameters, enables one to estimate the total number of tumor cells as well as any variation in number after therapy.

2. To determine the dynamics of cell cycle and proliferation kinetics and the effects of various antitumor drugs. The time course behavior of the cell age, cell size, and DNA content distributions of a tumor cell population is characterized by a discrete-time kinetic model employing computer techniques, and its implications to the design of therapeutic strategies are investigated.

3. To study by the formulation of metabolic reaction kinetics inhibitory activities of a number of antitumor drugs on specific enzymes in biosynthetic pathways, and to provide a better basis for optimum schedule of a drug or combinations of drugs for the treatment of leukemias and other forms of cancer.

Methods Employed

1. A multicompartment model for the cell cycle and proliferation kinetics was developed by treating each cell cycle phase as a separate compartment, consisting of compartments G_1, S, G_2 and M on the proliferating pool as well as compartment G_0 for the nonproliferating pool. The framework on the mathematical model was set up to describe the migration of cells between phases, the normal and drug induced mechanisms of cell loss that may occur within each phase, the normal and drug induced mechanisms of transit times in each phase, and the mechanisms of splitting (δ) mitotic daughter cells between proliferating and nonproliferating cells. Particular metabolic reactions that are specific to a phase may also be included (to describe biomarker kinetics). The general validity of the CELL KINETIC MODEL has been verified by successfully simulating the behavior of a number of experimentally investigated tumor systems, both free-growing and under the influence of drugs, including: L1210 leukemic mice (Cancer Res., 27: 2381-2385, 1967), Ehrlich's ascites tumor (Cancer Res., 29: 1527-1534, 1969), DMBA induced breast cancer (Cancer 28: 1478-1499, 1971) and a solid tumor (Cancer Res. 29: 1527-1534, 1969).

2. The CELL KINETIC MODEL has been used to simulate the growth behavior of tumors under drug treatment and to simulate the distribution and synthesis pattern of polyamines in the spleen of mice as a biomarker for L1210 leukemic cells (Cancer Res. 33: 159-163, 1973).

3. A discrete-time kinetic model was previously developed, based on the state equations with its state vector consisting of variables representing cells in a particular compartment for cell age, cell size, and cell DNA content. Further analysis on this MODEL was carried out in reference to variation of the kinetic parameters during the proliferation of Ehrlich ascites tumor (Virch. Archiv. Abt.B Cell Pathology 11: 1-10, 1972).

4. Analytical studies on control characteristics of allosteric enzymes involved in branched biosynthetic and amphibolic pathways have been continued. Compensatory feedback control in E. coli carbamyl phosphate synthetase was characterized quantitatively by nonlinear rate equations. The general framework of allosteric interactions in regulatory enzymes and substrate-modifier reaction will be expanded to deal with the analysis of drug inhibition in the biosynthesis of key metabolites in tumor growth kinetics.

Major Finding

1. Tumor Cell-Biomarker Kinetics. The CELL KINETIC MODEL with kinetic parameters (the cell cycle time and the rate of cell loss) expressed as a function of tumor cell population size represented the growth kinetics of various forms of animal tumors. The MODEL enables one to predict and analyze various properties of the cell cycle, including the number of cells in each phase at any point in time, the actual drug effects, the specific growth rate and the growth fraction, etc. It also provides a framework to predict possible behaviors of the tumor under synergistic effects of two or more drugs.

The L1210 tumor-polyamine system was characterized by integrating the CELL KINETIC MODEL with the rate equations for biosynthesis of polyamines. The results compared with experimental data were rather satisfactory, although discrepancy between them is still significant. However, further improvement on this integrated MODEL should result in the development of a useful tool in relating the concentration of biomarker experimentally (or clinically) measured to the number of tumor cells.

2. Cell Age, Cell Size, and Cell DNA Content Distributions. The discrete-time kinetic model previously developed has been analyzed further, and used to characterize the dynamics of the cell age and DNA content distributions during the proliferation of Ehrlich ascites tumor and to predict associated variation of its cell age distribution. Appearance of the second peak in the experimental cell volume distribution with the aging of the tumor (Virch. Archiv. Abt. B Cell Pathology, 11: 1-10, 1972) was interpreted that with the aging of the tumor there is a transition of the proliferating cells to the nonproliferating compartment and an accumulation of cells in the resting states. Accordingly, in this study this interpretation is used as a basis for an assumption that the resting cells are originated in the latter part of their cell cycle (G_2 - G_0 transition). Further study on this assumption including various modes of the arrested or resting state, is under way by computer simulation of the MODEL.

3. Metabolic Control Mechanisms. The analysis of control activity of E. coli carbamyl phosphate synthetase shows that sustained oscillations as well as the amplitude-modulated oscillations may exist in compensatory feedback control. As a two-input (activator and inhibitor) nonlinearity is approximated by two single-input nonlinearities, the control characteristics reveal a memory-type characteristic, which is long suspected in enzyme actions. Although carbamyl-P synthetase was not considered to be a hysteretic enzyme (J. Biol. Chem., 245: 5788-5799, 1970), this enzyme may express such characteristic under certain biological situations.

Significance to Biomedical Research and the Program of the Institute

A mathematical model developed for tumor-biomarker interactions characterizes well the kinetic relationship between L1210 leukemic cells and polyamine. The clinical implications of the model would be greatly enhanced when the size or proliferation rate of tumor is estimated by this model using polyamine readily measurable in patients with various forms of cancer and leukemias. The model may be applicable to other types of tumor cells and corresponding biomarkers, which requires different functional forms of kinetic parameters that represent the control of tumor cell proliferation.

A discrete-time kinetic model can describe and predict the time course behavior of the cell age, cell size, and DNA content distributions and also the movement of labeled cohort through the cell cycle. Such characteristics are useful to gain insights into the dynamics of cell cycle kinetics and the effects of antitumor drugs on tumor growth in terms of cell kinetic parameters.

Proposed Course

1. Efforts will be continued to investigate the kinetic behavior of cell cycle and proliferation of S-180 by the CELL KINETIC MODEL.

2. Specific efforts will be continued to integrate the proliferation kinetics of tumor cells and the activities of enzymes responsible for biomarker syntheses and to characterize the effects of antitumor agents on the tumor-biomarker interactions. The model of the tumor-polyamine system will be modified to allow its future extension to other types of biomarkers for cancer. Different (new) hypotheses and assumptions will be proposed and investigated in relation to tumor growth mechanisms as a function of tumor cell size and substances responsible for cellular regulation.

3. The discrete-time kinetic model will be applied to the investigation of the kinetic behavior of solid tumors. The effects of antitumor agents on the cell size and cell DNA content distributions will be investigated by including a state vector feedback in the model, which then becomes a discrete-time feedback control system. Analytical techniques developed of discrete-data control systems will be applied to study: (a) the stability of the system to determine the time course behavior and various relations of kinetic parameters to a drug or combinations of drugs, and (b) optimal or suboptimal schedule of drug treatment.

4. An attempt will be made to develop a kinetic equation which characterizes inhibitory activities of antitumor agents on DNA polymerases. Patterns of the interaction of drugs that inhibit steps of biosynthetic pathways of key metabolites will be analyzed; and kinetic studies of the interactions of two antitumor drugs will be carried out in relation to the growth of tumor cells and the rate of metabolite synthesis.

Honors and Awards:

Kwang B. Woo

1. Invited by the School of Science and Technology, Southern Illinois University to present a seminar on "Tumor Cell-Biomarker Kinetics and Cancer Chemotherapy." October, 1973.

2. "The Kinetics of L1210 Leukemic Cell Proliferation." Presented at the Tenth International Conference on Medical and Biological Engineering in Dresden, GDR. August, 1973.

3. "Compensatory Feedback Control in Biochemical Reactions: Control Activity of E. coli Carbamyl Phosphate Synthetase." Presented at the IFAC Symposium on Dynamics and Control in Physiological Systems in Rochester, New York. August, 1973.

4. "A Quantitative Model for the Cell Kinetics of a Proliferating Cell Population and Its Application to Movement of Labelled Cohort" presented at the Twenty-sixth Annual Conference on Engineering in Medicine and Biology in Minneapolis, Minnesota. September, 1973.

5. "The Variation of Cell Loss and Its Effects on Tumor Cell Proliferation Kinetics". Presented at the Twenty-sixth Annual Conference on Engineering in Medicine and Biology in Minneapolis, Minnesota. September, 1973.

6. "Analysis of the Effect of Antitumor Drugs on the Kinetics of Tumor Cell-Biomarker Relationship". Presented at the Thirteenth Hanford Biology Symposium on "The Cell Cycle in Malignancy and Immunity", Richland, Wash. October, 1973.

Publications:

1. Ghista, D. N., Patil, K. M., Gould, P. and Woo, K. B.: Computerized left ventricular mechanics and control system analyses models relevant for cardiac diagnosis. Comput. Med. Biol. 3: 27-46, 1973.

2. Woo, K. B. and Huang, H. M.: Compensatory feedback control in biochemical reactions: Control activity of E. coli carbamyl phosphate synthetase. In: Regulation and Control in Physiological Systems, Iberall, A. S. and Guyton, A. C. (eds.), Pittsburgh, Instrument Society of America, pp 22-26, 1973.

3. Woo, K. B. and Wiig, K. M.: Analysis of the effect of antitumor drugs on the kinetics of tumor cell-biomarker relationship. In: The Cell Cycle in Malignancy and Immunity, Hampton, J. C. (ed.), the AEC Symposium Series, in press.

4. Kim, M., Bahrami, K. and Woo, K. B.: A discrete-time model for cell age, size and DNA distributions of proliferating cells and its application to movement of labelled cohort. IEEE Trans. Biomed. Eng., in press.

SUMMARY REPORT

DRUG RESEARCH AND DEVELOPMENT (DR&D)

DIVISION OF CANCER TREATMENT (DCT)

Drug Research and Development has as its primary task the search for and development of new and potentially more effective antitumor agents, as well as the development of better methods of drug selection and prediction of optimal therapy with new and old drugs and drugs combined with other treatment modalities. With respect to the Division of Cancer Treatment (DCT) Linear Array, the organization is responsible for all preclinical work in the development of drugs except that concerned with toxicology and pharmacology.

The DR&D program includes both in-house laboratory operations and contracts with pharmaceutical and chemical companies, research institutes and academic institutions, with the major portion of the funding directed toward the contract-supported activities. The program depends heavily on additional collaboration with research grantees, other independent investigators throughout the world, and hundreds of industrial companies not involved in contracts. The program activities cover the following specific areas:

1. Synthesis of new chemical agents.
2. Procurement and resynthesis of bulk chemicals and drugs for preclinical and clinical evaluation.
3. Development of new agents from fermentation, plant and other natural products.
4. Development and production of clinical dose forms for clinical trial.
5. Quality control for bulk and formulated drugs.
6. Primary screening and evaluation of new materials.
7. Bioassays, both *in vivo* and *in vitro*, to monitor the purification of fermentation and other natural products.
8. Study of optimal drug routes and regimens, including therapy with combinations of drugs and combined modalities.
9. Development of new and improved laboratory methods for discovering, evaluating and utilizing potential anti-tumor agents.
10. Investigation of mechanisms of action and resistance of drugs of clinical interest.
11. Animal procurement, distribution and disease control.
12. Procurement of radiolabeled drugs for clinical pharmacologic and experimental studies.
13. Development and operation of data processing systems for both biological and chemical information.
14. Publication of the journal, *Cancer Chemotherapy Reports*.

Major changes in the input and screening of new materials occurred during the past year. The Division of Cancer Treatment decided to increase the input of new synthetic compounds to the screen in order to accommodate the larger number of materials desired to be screened throughout the world as a result of the increased interest generated by the National Cancer Act. To evaluate a larger number of materials without an increased screening effort, the methodology previously described as the "mini-screen" was adapted for large-scale screening. In essence this sytem utilizes a smaller number of animals and only one dosage schedule rather than the two schedules previously used. As a result of this change in methodology and an active effort to obtain new synthetic compounds, the program was successful in obtaining a large number of compounds as shown in Table 1. It may be noted that the input of new synthetic compounds more than doubled from 1972 to 1973. The increase in the number of IND applications filed in 1973 does not bear any relation to that input number since it requires, on the average, four years from input to the filing of an IND application. That increase does reflect a general speedup in moving materials toward clinical trial as well as materials that progressed very rapidly from input to clinical trial because of prior clinical data (Cytembena, Ftorafur, and Asaley).

The *in vivo* screening effort continued to increase in 1973 in order to accommodate the increased input of compounds (Table 2). The increase was relatively small in spite of the rather large increase in synthetic compounds because of the change from two dosage regimens to one in the mini-screen procedures. The changes in procedure must still be evaluated in terms of relative yield of active materials. Initial impressions suggest that the yield may be lower, and this question is under intensive scrutiny. Suffice it to say, however, that the change in procedure did allow a much larger number of compounds to be tested. If it should become necessary to test new compounds more intensively than is done with the mini-screen, it would not be possible to maintain the level of input that is currently visualized. One cannot at this point determine the direction that will be taken in terms of input levels for the coming year since the above questions will play a very direct role in that decision.

Concomitant with the increased input of synthetic compounds was a decision to utilize activity or lack thereof in the four major systems, L1210, P388, B16 and Lewis lung as a measure of the relative priority to be given a new compound with activity in one of these systems. Several analyses have been carried out to determine the best way of utilizing relative activity in each of these systems as a method of judging the priority of a new compound, but no final decision has as yet been made on the specific method of evaluation. It is clear, however, that activity in B16 melanoma or Lewis lung carcinoma appears to provide a much higher level of prediction for activity in solid tumors of man than activity in either L1210 or P388 alone. Thus evaluations are carried out in all four of these systems for any compounds showing activity in either of the leukemia systems which are still used as the primary screens.

As a result of the screening and related drug development efforts, eight materials passed Decision Network 2A in 1973. That is, they demonstrated sufficient biological activity and chemical or other uniqueness to warrant the effort in dose formulation development, etc., leading to preclinical toxicology and clinical trial. These materials are listed in Table 3.

The coordinated preclinical drug development work in 1973 resulted in thirteen IND applications (non-endocrine) being filed, signifying the initiation of clinical trials. These materials are listed in Table 4.

Other Selected Highlights

1. As a part of the overall shift of the Division of Cancer Treatment's program toward combined modalities, a beginning has been made in the development of experimental systems. The Lewis lung carcinoma, of course, has been used for many years as a surgical adjuvant system and it will continue to be used in that way. Other systems are currently being evaluated to determine if they can be utilized for combined radiotherapy and drug therapy. It is expected that a much greater effort in this area will be exerted during the coming year.

2. A new methodology has been developed for the preparation of plant extracts for initial testing, consisting of an ethanol extraction followed by a preliminary fractionation prior to screening. This procedure has resulted in the removal of much extraneous material, the concentration of activity into two fractions, and the production of an apparently much higher yield of active extracts. This methodology, initially tested on a pilot scale, will likely be used for all plant materials in the next year.

3. Significant progress has been made in identifying families of plants in which collections will be emphasized because of the relatively high yield, and other families in which collection will be greatly reduced or dropped because of a relatively small yield. This method has been possible because of a newly developed computer analysis.

4. The total synthesis of daunomycin and adriamycin should be possible in the foreseeable future in view of the recent success in synthesizing daunosamine and the chemical coupling of daunosamine to daunomycinone.

5. *Cancer Chemotherapy Reports* has continued to be an actively utilized primary and specialized journal, with a total circulation of approximately 5300. During calendar year 1973 four issues of the primary journal (Part 1) and three issues of program information (Part 3) were published. The number of manuscripts received for Part 1 has increased by almost 50% in the past two years indicating considerable interest on the part of the scientific public publishing in this journal.

Organizational Changes

During the past year a major reorganization of Drug Research and Development took place. The Program Analyses Branch was abolished and its sections moved elsewhere. The two data processing activities were moved into the Drug Evaluation Branch and Drug Development Branch in accordance with the area of activity. The Literature Research Section was transferred to the Cancer Therapy Evaluation program, and the Publication Section was moved into the Office of the Associate Director, DR&D.

The Laboratory of Experimental Chemotherapy was created and currently includes two sections; the Tumor Biology Section incorporates some activities from the Office of the Associate Director and some from the Screening Section, Drug Evaluation Branch. In addition the Immunochemotherapy Section was created from the original Immunotherapy and Immune Response Modifiers Section of the Drug Evaluation Branch. It is expected that at least two additonal sections will be activated within the Laboratory of Experimental Chemotherapy during the coming year in areas of biochemistry and bio-organic chemistry. We should then have a very active laboratory to develop and pursue many basic problems of experimental chemotherapy. During the formative stages of the Laboratory of Experimental Chemotherapy, Dr. Abraham Goldin has agreed to function as Acting Chief in addition to his functions as Associate Chief for Laboratory Research, DR&D. The present organization of Drug Research and Development is as follows:

Drug Research and Development

Associate Director for Drug Research and Development -
  Saul A. Schepartz

  Associate Chief for Laboratory Research - Abraham Goldin
  Special Assistant to Associate Director
    for Drug Research and Development - Barbara R. Murray
  Mammalian Genetics and Animal Production Section - Samuel M. Poiley, Head
  Publications Section - Pamela W. Hutchins, Head

  Drug Development Branch - Harry B. Wood, Jr., Chief

    Chemical and Drug Procurement Section - Robert R. Engle, Head
    Chemical and Drug Records Section - Robert B. Ing, Head
    Clinical Drug Distribution Section - J. Paul Davignon, Head
    Drug Design and Chemistry Section - John S. Driscoll, Acting Head
    Data Processing and Computer Systems Development
      Section - Sidney Richman, Head
    Natural Products Section - Jonathan L. Hartwell, Head
    Scientific Records Section - George F. Hazard, Head

  Drug Evaluation Branch - John M. Venditti, Chief

    Automated Information Section - Mary Macdonald, Acting Head
    Biochemistry Section - Florence R. White, Head
    Molecular Biology and Methods Development Section - Vincent Bono, Head
    Screening Section - Betty J. Abbott, Head

  Laboratory of Experimental Chemotherapy - Abraham Goldin, Acting Head

    Immunochemotherapy Section - Enzo Bonmassar, Head
    Tumor Biology Section - Randall K. Johnson, Acting Head

Table 1

Input and Output of

Drug Development Program

Materials Submitted	1966	1967	1968	1969	1970	1971	1972	1973
	Calendar Year							
Synthetic & crystalline natural products	8,008	7,413	10,915	9,820	8,521	10,151	15,770	34,143
Crude fermentation products	4,284	4,982	4,256	2,184	2,172	1,215	2,712	2,550
Crude plant products	3,620	6,253	4,245	5,109	3,499	3,015	10,434	11,661
Animal products		446	641	757	587	1,203	1,669	2,689
Total	15,912	19,094	20,057	17,870	14,779	15,584	30,585	51,334
IND applications filed	3	4	5	5	6	8	5	13

Table 2

Screening Tests

	Calendar Year					
	1968	1969	1970	1971	1972	1973
L1210 Lymphoid Leukemia	107,445	80,545	79,892	102,424	103,681	133,481
P388 Lymphocytic Leukemia	14,903	43,223	42,270	53,449	180,027	198,711
Walker Carcinosarcoma 256	44,563	20,328	10,247	4,368	2,770	--
B16 Melanoma	286	1,240	1,342	1,423	6,028	15,989
Lewis Lung Carcinoma	244	925	3,033	2,803	4,141	2,441
Other Tumors	774	426	504	1,780	6,458	5,493
Total *In vivo*	168,215	146,687	137,288	166,247	303,105	356,115

Table 3

Compounds Passing Decision Network 2A

NSC No.	Compound Name	Source
51,143	1H-Pyrazolo[2,3-*a*] imidazole	Synthetic
73,754	Uracil, 5-[(2-chloroethyl)(2-fluoroethyl)-amino]-6-methyl-	Synthetic
124,921 D		
154,020	1,4,5,6,8-Pentaazaacenaphthylene, 3-amino-1,5-dihydro-5-methyl-1-β-*D*-ribofuranosyl-	Synthetic
165,563	Bruceantin	Plant
167,780	*L*-Leucine, *N*-[*N*-acetyl-4-[bis(2-chloroethyl) amino]-DL-phenylalanyl]-, ethyl ester	Synthetic
167,781	Benzamide, *N*-[bis(1-aziridinyl)phosphinyl]-2,5-diiodo-	Synthetic
--	Coralyne sulfoacetate*	Synthetic

* Synthetic derivative of plant product

Table 4

IND'S Filed

NSC No.	Compound Name	Source
15,200	Gallium nitrate	Synthetic
71,851	9*H*-Purine-6-thiol, 2-amino-9-(2-deoxy-α-*D*-*erythro*-pentofuranosyl-	Synthetic
77,037	d-Tetrandrine	Plant
83,265	*L*-Alanine, 3-(tritylthio)-	Synthetic
104,801	Cytembena	Synthetic
118,994	Diglycolaldehyde, -(hydroxymethyl)-α'-(6-hydroxy-9*H*-purin-9-yl)-	Synthetic
135,758	2,5-Piperazinedione, 3,6-bis(5-chloro-2-piperidyl)-, dihydrochloride	Fermentation
139,105	Ethanesulfonic acid, compd. with α-[2-chloro-4-(4,6-diamino-2,2-dimethyl-*s*-triazin-1(2*H*)-yl)phenoxy]-*N*,*N*-dimethyl-*m*-toluamide	Synthetic
143,769	Methanol extraction residue (MER) of BCG	Fermentation
145,668	*O*2,2'-Cyclocytidine, monohydrochloride	Synthetic
148,958	Ftorafur	Synthetic
167,780	*L*-Leucine, *N*-[*N*-acetyl-4-[bis(2-chloroethyl)-amino]-DL-phenylalanyl]-, ethyl ester (Asaley)	Synthetic
B116,327	BCG (Tice Strain)	Fermentation
B116,328	BCG (Pasteur)	Fermentation

Total IND's Filed 13
(Non-endocrine)

Serial No. NCI 3575
1. Office of Associate Director For Drug Research and Development, Division of Cancer Treatment, NCI
2. --
3. NCI - Building 37 and Microbiological Associates, Bethesda, Maryland

PHS-NIH
Individual Project Report

July 1, 1973 through June 30, 1974

Project Title: Chemotherapy research including fundamental biological and biochemical investigation of antitumor agents

Previous Serial Number: Same

Principal Investigator: Abraham Goldin

Co-Principal Investigators: A. W. Schrecker, R. K. Johnson, E. Bonmassar, J. M. Venditti, O. C. Yoder

Other Investigators: D. P. Houchens, V. V. Subba Reddy, B. Garibjanian, A. A. Ovejera, I. Umezawa, T. Inouye

Cooperating Units: G. Cudkowicz, State University of New York at Buffalo; J. M. Venditti and Staff, Drug Evaluation Branch, DCT, NCI; H. B. Wood, Jr., R. R. Engle, J. Discoll and J. Hartwell, Drug Development Branch, DCT, NCI; J.A.R. Mead, Experimental Therapeutics, DCT, NCI; R. Gallo, Human Tumor Cell Biology Branch, DCT,NCI; N. Mantel, Biometry Branch, NCI; M. Sporn, Carcinogenesis, NCI; T. Borsos, B. Zbar and Staff, Biology Branch, NCI; P. Carbone, Medical Oncology, NCI; S. K. Carter, Cancer Therapy Evaluation, DCT, NCI; Y. Ikawa, Viral Leukemia and Lymphoma Branch, NCI; H. E. Skipper, F. M. Schabel, Jr. and Staff, Southern Research Institute; I. Kline, S. Vadlamudi and M. Kende, Microbiological Associates, Inc.; I. Wodinsky, A. D. Little; E. Frei, III, Children's Cancer Research Foundation, Boston; F. M. Huennekens, Scripps Clinic and Research Foundation, La Jolla; A. DiMarco, Farmitalia, Milan, Italy; A. Nicolin, University of Milan, Italy; S. Garattini, Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy

Man Years
Total: 11-1/2
Professional: 10
Others: 1-1/2

Project Description:

The scope of this project in the Office of the Associate Director, Drug Research and Development is being altered, since the Laboratory of Experimental Chemotherapy was established during the year and two of the sections, the Tumor Biology Section and the Immunochemotherapy Section were activated. The year has thus been one of transition and the activities of these two sections will be transferred from this project.

Objectives:

I. To contribute to the planning and coordination of the chemotherapy research program of Drug Research and Development and application to drug development and total program.

II. To conduct fundamental investigations on special problems pertaining to potential and established clinical cancer chemotherapeutic agents; to obtain biological and biochemical knowledge concerning chemical agents capable of affecting malignant growth; to determine means for improvement of therapy with existing drugs; to investigate the influence of structural modification of drugs on the mechanism of antitumor actions, to provide basic information of pertinence to the clinical use of such agents; to study specific problems encountered during the use of these agents in the clinic.

III. To collaborate with the Drug Evaluation and Drug Development programs of the Drug Research and Development and other preclinical programs in the Division of Cancer Treatment, in structure-activity studies and in the exploration of new and potentially useful biological and biochemical methodology for evaluation and improvement of drug therapy and in special problems and new approaches.

IV. To collaborate with the clinical program, Division of Cancer Treatment in areas interrelating preclinical and clinical investigations. This encompasses studies of the predictability of model tumors for the clinic, investigation of combination chemotherapy and combination modalities and joint planning and conduct of problems of specific pertinence to the clinic.

V. Conduct of international activities including maintenance of chemotherapy offices in Europe and Asia, arrangements for acquisition of test materials, screening of new agents abroad and surveillance for and acquisition of new antitumor agents. Included in this area is an effort aimed at development of standards for world-wide antitumor screening methodology and interchange of screening and drug evaluation data. Also collaborative research programs are conducted on compounds and problems of specific interest with scientists of various countries including the Soviet Union, Italy, Japan and others. A contract for collection, purification and testing of plant extracts is also being conducted in Israel.

This program is planned so that it will be highly responsive to the needs of the preclinical and clinical chemotherapy program of the Cancer Institute. In addition to the laboratories in our building the Kenwood Laboratories staffed by Microbiological Associates professional and technical personnel has served as a collaborative laboratory resource for the research staff of this program. The chemotherapy research program collaborates closely with the ongoing programs in Drug Evaluation, Drug Development, Immunology, Organic Chemistry and Pharmacology. The program serves as a focal point for the interdigitation of chemotherapy research including the DR&D screening, developmental and basic chemotherapeutic investigations with collaborating DR&D contractors and clinical programs of NCI and other institutions. It maintains close relationships with medical schools, pharmaceutical houses, university and research institutes both in the United States and abroad in areas pertinent to chemotherapy.

Methods Employed: Standard laboratory procedures were employed.

International Activities

Progress is being made in information exchange on most recent developments in chemotherapy, on the procurement of synthetic agents and natural products for screening and in the identification of new compounds of potential interest for chemotherapy.

Close liaison is being maintained with investigators in leading institutions including universities, medical schools and pharmaceutical houses abroad. There is active contact with the Chemotherapy Committee of the International Union Contra Cancrum, the European Organization for Research and Treatment of Cancer (EORTC), the World Health Organization in Geneva and the International Agency for Research in Cancer of the World Health Organization. The Division of Cancer Treatment Chemotherapy Offices at the Institut Jules Bordet, Brussels, Belgium and at the Cancer Institute, Tokyo, Japan are making progress in providing a means for close collaboration with the preclinical and clinical cancer chemotherapy programs of Europe and Asia. The chemotherapy offices are functioning successfully in the gathering and exchange of scientific knowledge for the further development of cancer chemotherapy. They are significantly increasing the input of new drugs and screening data into the chemotherapy screening program of the National Cancer Institute and stimulating collaboration in the study of new anticancer drugs. The chemotherapy offices are providing a means for the continuous review, abstracting, and where necessary, translating of significant foreign reports.

Dr. Omar Yoder, serving as an NCI Division of Cancer Treatment officer at the European Office, is making excellent progress in furthering the overall collaboration in Europe. He is expanding and maintaining contacts with pharmaceutical houses, chemical industry and universities in the acquisition of new compounds for screening and in the exchange of preclinical and clinical information. In addition to his activities at the Jules Bordet Institut he has been making visits to important institutions in the various countries of Europe, working in collaboration with the European Organization for Research and Treatment of Cancer (EORTC) as well as the other European organizations. This has resulted not only in enlarging the contacts for materials for screening but also in providing extensive information on drugs and modalities of therapy of clinical interest. The Jules Bordet Institut is serving as the data and coordinating center for the E.O.R.T.C. which gives our office an excellent tie-in with all of the preclinical and clinical chemotherapy activities in Western Europe. The screening program at the Jules Bordet Institut is exceeding the projected figures for the screening of new antitumor agents. The materials tested in the screening program are collected by the DCT office in Europe. A screening program is also functioning successfully at the Mario Negri Institute in Milan for the testing of compounds collected primarily in Southern Europe. A chemotherapy testing program is being instituted at the Chester Beatty Institute in England and assistance is being provided in the setting up of a screening program at the Cancer Institute in Dublin, Ireland. A program on plant collection, extraction and screening at the Weizmann Institute in Rehovoth, Israel is making progress.

The screening program instituted last year at the Cancer Institute in Tokyo as part of the "Anticancer Drug Evaluation Center" is making good progress in the screening of candidate agents. The materials that are being tested in the screens are being collected primarily from pharmaceutical houses and universities in Japan. The data collected in the screening programs in both Europe and Asia are entered into the Drug Research and Development computer program. The DCT offices in both Brussels and Tokyo continue to publicize extensively the DCT program in the various countries of Europe and Asia.

As part of the U.S.-U.S.S.R. Cooperative Medical Agreement a preclinical chemotherapy team visited the preclinical facilities in the Soviet Union in order to broaden and facilitate exchange of information and for the expediting of the overall collaborative effort. A collaborative program has been instituted for the interchange and evaluation of Soviet and American drugs. This has been functioning well and data have now been obtained which will lead to a number of joint publications.

The plans for an International Conference on Cancer Chemotherapy Screening organized jointly by the U.S. and U.S.S.R. are proceeding well. The meeting will be held under the auspices of the World Health Organization in Geneva and about twenty countries will be represented.

Visits were made by A. Goldin as follows:

(1) Drs. C. Gordon Zubrod and A. Goldin attended the Tenth Anniversary of the Mario Negri Pharmacology Institute in Milan at which Dr. Zubrod presented the primary lecture and Dr. A. Goldin participated in discussion. This provided an opportunity for an on-site visit to this institute which is undoubtedly a leading pharmacology institute in Italy.

Discussions concerning adriamycin were carried out at the Mario Negri Institute, at the Tumor Institute in Milan and at the Farmitalia Pharmaceutical Company. Our NCI representative at the E.O.R.T.C. headquarters in Brussels, Dr. Omar Yoder, attended the meetings at which there was also discussion of the strategy for compound collection in Western Europe. Dr. Aurelio Napolkov was present in Milan and in his capacity at that time as Chief of Cancer Control at WHO there was discussion of the impact of the National Cancer Program Plan and the Soviet-U.S. agreement on other countries. The possible role of WHO in the planning of cancer research was also discussed.

(2) As part of the international collaborative effort in cancer chemotherapy the Division of Cancer Treatment, NCI, co-sponsored an International Course on Tumor Cell Kinetics and Antineoplastic Chemotherapy in Buenos Aires, Argentina. The organizing committee in Buenos Aires consisted of Dr. Roberto A. Estevez, Chief of Oncology Service, Hospital Militar Central and a vice-president of the International Union Against Cancer. Dr. A. Goldin was the official NCI representative, responsible for the co-organization of the course. The faculty was international and the students attending the course were at the post-graduate level or more advanced, with specialization in clinical oncology and antitumor chemotherapy and background in tumor cell kinetics. They came primarily from Argentina, Ecuador, and Brazil and evidenced a strong interest in cancer chemotherapy both at the preclinical and clinical levels. South American cancer chemotherapy has strong potential, and active collaboration and cooperation with the national program in the United States could prove of great value.

(3) Drs. S. A. Schepartz, A. Goldin, G. R. Pettit and N. Ferguson visited the leading cancer research institutes in the Soviet Union in Moscow, Riga, Leningrad, Kiev and Yerevan. The visits were eminently successful in providing background pertaining to the status and recent developments in preclinical cancer chemotherapy, and offering an opportunity for personal associations which may contribute immeasurably to the future cooperation in cancer chemotherapy with the U.S.S.R. Considerable emphasis was placed on the visit to the Institute of Experimental and Clinical Oncology in Moscow which is undoubtedly the primary cancer chemotherapy institute in the U.S.S.R. and which appears to be rapidly assuming leadership as the central agency for preclinical as well as clinical cancer chemotherapy in the Soviet Union. Specific items relating directly to the U.S.-U.S.S.R. agreement include the following:

(a) Arrangements were made for additional interchange of antitumor agents for clinical trial.

(b) Summary information sheets on nine Soviet drugs were provided.

(c) Arrangements were made for a new Soviet antibiotic antitumor agent carminomycin to be made available to the National Cancer Institute.

(d) Arrangements were made for the interchange of a number of experimental tumors.

(e) Discussions were held concerning the further inter-visits of preclinical cancer investigators.

(f) There is a great reservoir of synthetic compounds and natural products in the Soviet Union that could be the subject of antitumor chemotherapeutic evaluation. Their availability for screening was discussed.

(g) The setting up of a large scale screening program in the Soviet Union similar to the one at the National Cancer Institute, U.S.A. appears feasible and was encouraged.

(h) Mechanisms for maximum exchange of data covering all of the compounds included in the exchanges were explored and a basis provided for the establishment of such procedures.

(i) Discussion was held concerning the importance of extending the U.S.-U.S.S.R. Agreement to incorporate pertinent activities of the Academies of Science in both countries.

(j) The plans for the establishment of an Institute of Biotesting of chemical compounds within the Soviet Ministry of Medical Industry were discussed.

(k) Further plans were made for a world meeting on screening and drug evaluation.

(4) Participation in "Symposium on Clinical Pharmacology With Special Reference to Cancer" at the Institut Jules Bordet, Brussels. The symposium was held under the auspices of the European Organization for Research on Treatment of Cancer (E.O.R.T.C.) with the support of the International Agency for Research on Cancer, Lyon, France. A. Goldin chaired a session on "Design and Screening of New Drugs", in addition to presentation of a paper and general participation. Subjects covered included preclinical evaluation procedures, design and development of anticancer agents, tests for anti-metastatic activity, relationship between cancer in animal and man, immunotherapy, methodology of clinical trials and pharmacokinetics and metabolism of anticancer agents. The staff was international in makeup and the participants who attended were from a number of countries in Europe.

(5) A visit was conducted by Dr. A. Goldin along with Drs. S. Carter and S. Perry to the Japanese Cancer Institute to confer regarding activities relating to our cancer chemotherapy office in Tokyo. A visit was made to Drs. T. Ichikawa's and Koyama's group at the Tokyo First National Hospital, for detailed discussion of bleomycin. A visit was made to Drs. Sakurai, Tsukagoshi and Hoshino at the Cancer Institute (Japanese Foundation for Cancer Research) for discussion of problems

pertaining to the newly formed Cancer Chemotherapy Center. This was important since Professor Yoshida who had been the Head of the Institute and the Project Director on the contract had recently died. A visit was made to the Taisho Pharmaceutical Company Laboratories where they have a comprehensive program involving various aspects of pharmacologic and toxicologic testing of new drugs. A meeting was held at the Cancer Institute at which there was a detailed review of neocarzinostatin. In attendance were representatives of the various groups conducting the preclinical and clinical investigations with this drug.

Dedication ceremonies were held for the new Cancer Chemotherapy Center at the Japanese Cancer Institute. The dedication ceremonies were highly impressive and attended by leading preclinical and clinical investigators from thoughout Japan. It was attended by Prince and Princess Hitachi who have been staunch supporters of the Japanese Cancer Institute. A. Goldin had the responsibility for giving the congratulatory dedication address. The dedication ceremonies were followed by a scientific meeting in which we participated.

An additional meeting was held at the National Cancer Center Hospital under the auspices of Dr. K. Kimura who is a leading cancer chemotherapy clinical investigator in Japan.

A meeting was held concerning the contract at the Cancer Institute at which it was announced that Dr. Sakurai had been appointed Head of the Cancer Chemotherapy Center. It was also determined that he would replace Professor Yoshida as Project Officer on the contract. The meeting provided an opportunity for discussion of future plans and program for the contract.

(6) A trip was made to Brussels, Belgium; Rijswijk, the Netherlands; Athens, Greece and Riga, U.S.S.R. The visit to Brussels was to the Institut Jules Bordet Chemotherapy Office to confer concerning recent progress. This was considered important at that time since the ongoing screening program employing NCI protocols for leukemia L1210 and leukemia P388 was being incorporated into the NCI program on a formal basis and plans were being made to also incorporate the B16 melanoma as part of the program.

A seminar was presented at the Radiobiological Institute in Rijswijk, the Netherlands and discussions were conducted with Drs. L. M. van Putten and D. W. van Bekkum. The Radiobiological Institute has relatively new buildings housing advanced radiological, biochemical, pharmacologic and biologic equipment and they are conducting highly important work in various areas of considerable interest to our program. In view of Dr. van Bekkum's central position in the E.O.R.T.C. an opportunity was afforded for further discussion of the relationship of E.O.R.T.C. program to the National Cancer Institute therapy program.

The VIIIth International Congress of Chemotherapy was held in Athens, Greece. At the International Congresses of Chemotherapy there is increasing emphasis on various aspects of cancer research and cancer chemotherapy. There were approximately 1500 delegates at this Congress with a broad range of representation from various countries of the world. A. Goldin co-chaired a panel on "*In Vitro* Systems for the Detection of New Anticancer Agents". In addition to papers of fundamental scientific interest, papers were presented on a number of drugs of preclinical and clinical interest.

A visit was made to the Institute of Organic Synthesis in Riga to participate in a joint symposium with preclinical and clinical investigators of the U.S.S.R. and Japan on the Soviet drug Ftorafur and to participate in detailed discussions pertaining to cancer chemotherapy. Prior to the meetings on Ftorafur A. Goldin presented two talks at the Institute of Organic Synthesis: (1) Schedule Dependency of Drugs, (2) Combination Chemotherapy and Clinical Correlation. At the Ftorafur Symposium, A. Goldin presented a paper entitled "Screening of Antitumor Drugs in the U.S.A." and presented the U.S.A. chemotherapy data on Ftorafur. It was noted that the extent of collaboration of the Japanese and Soviet investigators in relation to new drugs for the treatment of cancer is quite comprehensive both at the preclinical and clinical levels. Professor S. Hiller who is the Head of the Institute of Organic Synthesis emphasized the importance and expressed a great interest in collaborative investigations with our program both on a scientist to scientist basis and through the joint U.S.-Soviet Medical Agreement. The Institute of Organic Synthesis is a very sophisticated institute. The staff is highly professional and enthusiastic and one is indeed impressed with the level of organic synthesis and the new building and equipment that is being devoted to biologic investigations. They are considering the possibility of setting up a modest screening program in cancer chemotherapy in coordination with the Institute of Oncology in Moscow.

(7) A visit was made with Drs. S. Schepartz, H. Wood and O. Yoder to the Mario Negri Institute in Milan where Professor Garattini had arranged a special meeting with a large number of industrial and academic suppliers throughout Italy in order to explain to them the NCI program and to hopefully induce them to submit compounds to the NCI program for screening. Approximately 125 people attended this meeting and the response was good. Following formal presentations in which we participated there were many discussions on an individual basis as well as general questions. This type of meeting was a very good format for encouraging the collaboration of investigators on a broad basis.

The visit also provided an opportunity to inspect the screening contract operation and the animal facilities, as well as to have discussions with scientists in the three major areas of interest of this Institute, namely, cancer chemotherapy, psychopharmacology and cardiovascular diseases.

We met with Dr. Rivalta and Professor Ghione and staff of the Pharmaceutical Division of Montecatini Edison in Milan concerning a potential contract between Farmitalia and NCI for the further development of analogs of adriamycin. It was considered that the company is genuinely interested in such a collaborative program and if some formal relationship could be established they expressed their willingness to exchange information on all work being done on analogs of adriamycin whether conducted under our support or not. It provided an opportunity for clarification of certain legal impediments and it was considered that the discussions contributed markedly toward the possibility of cooperation on a more formal basis. Since they have made great progress in the adriamycin area, it is felt that it would be in NCI's interest to work with them more closely, if at all possible.

An opportunity was also provided to visit Dr. DiMarco and his associates at the Cancer Institute in Milan where important work is being carried out on biological problems relating to adriamycin and its analogs. We have a close working relationship with Dr. DiMarco's group and this cooperation is continuing.

The following talks describing various aspects and results of our program were given abroad during the year by A. Goldin:

(1) "Antitumor Screening Systems" presented at the International Course on Tumor Cell Kinetics and Antineoplastic Chemotherapy, Buenos Aires, Argentina.

(2) "Importance of Dose Schedule and Route of Administration" presented at the International Course on Tumor Cell Kinetics and Antineoplastic Chemotherapy, Buenos Aires, Argentina.

(3) "Combination Chemotherapy" presented at the International Course on Tumor Cell Kinetics and Antineoplastic Chemotherapy, Buenos Aires, Argentina.

(4) "Aspects of the Preclinical Chemotherapy Program of NCI" presented in Moscow, Leningrad and Riga.

(5) "Preclinical Evaluation Procedures for New Anticancer Drugs" presented at the Symposium on Clinical Pharmacology with Special Reference to Cancer, Jules Bordet Institut, Brussels.

(6) "Congratulatory Dedication Address" at Dedication Ceremonies of the Cancer Chemotherapy Center, Japanese Cancer Institute.

(7) "Preclinical Investigation of Antitumor Agents" given at the Japanese Cancer Institute, Tokyo.

(8) "Preclinical Evaluation of Antitumor Agents" presented at Rijswijk, the Netherlands.

(9) "Importance of Determination of the Schedule Dependency of Drugs" presented in Riga, U.S.S.R.

(10) "Preclinical Investigations of Combination Chemotherapy

and Clinical Correlations presented in Riga, U.S.S.R.

(11) "Screening of Antitumor Drugs in the U.S.A." presented in Riga, U.S.S.R.

(12) "Screening Systems for New Antitumor Agents" presented at Mario Negri Institute, Milan, Italy.

Major Findings:

I. The Relationship of Structure to Antitumor Activity

The program relating structure to antitumor activity has been continued in collaboration with Drs. Wood, Johnson, Engle, Driscoll, Mead and Venditti. Areas in which structure activity studies have been conducted include actinomycin derivatives, adriamycin derivatives, detailed comparisons of fluorouracil and the Soviet drug ftorafur and folic acid antagonists.

II. Mechanism of Action of Antitumor Agents

A. Methotrexate Uptake. (A. W. Schrecker and F. M. Huennekens)

Study of the uptake of methotrexate by L1210 leukemia cells in culture, as influenced by the intracellular level of cyclic AMP. The rate of uptake and the final equilibrium concentrations in the cells are determined. The cells are preincubated with the dibutyryl derivative of cyclic AMP and/or with phosphodiesterase inhibitors such as theophylline or papaverine. The rationale for this study is that in malignant cells the level of cyclic AMP is generally lower than in normal cells, and that a decrease in the cyclic AMP level is often correlated with an increase in metabolite transport. Methotrexate enters cells by the same transport mechanism as tetrahydrofolate, an essential cellular metabolite, and it appeared of interest to find out whether the selective effect of methotrexate against leukemic cells is related to their cyclic AMP level.

This work is being carried out in the Department of Biochemistry at the Scripps Clinic and Research Foundation under the sponsorship of Dr. Frank M. Huennekens.

B. Enzyme Studies of Leukemia L1210 Sublines Resistant to Various Drugs (B. Akhtarzandi, with S. Vadlamudi, R. Johnson, J.A.R. Mead)

In continuation of a program attempting to relate enzyme activity to resistance it was found that a 5-fluorouracil resistant subline of leukemia L1210 showed no change in thymidylate synthetase, deoxycytidine kinase or dihydrofolate reductase activity. This would suggest that 5-fluorouracil resistance in this instance may not be caused by the inhibition of thymidylate synthetase activity but may be attributable to inhibition of some other enzyme, possibly at the phosphorylating stage.

C. Serum Enzyme Studies of AKR Spontaneous Leukemia. (with M. Kende)

In an attempt to develop chemical markers to determine status of disease during therapy, studies were conducted of 3 serum enzymes, alkaline phosphatase, lactic dehydrogenase and glutamic oxaloacetic transaminase in AKR mice. For leukemic AKR mice the alkaline phosphorylase level was 130% greater than in normal mice. The serum lactic dehydrogenase and glutamic oxaloacetic transaminase levels in the leukemic mice were about 300% and 250% higher respectively than in normal mice. Chemotherapeutic application of cyclophosphamide to the AKR leukemic mice resulted in appreciable decrease in enzyme levels particularly for lactic dehydrogenase and glutamic oxaloacetic transaminase, accompanying remission induction. It would appear that the enzyme levels reflect the antileukemic activity of cyclophosphamide and may serve as indicators of the progress of disease and efficacy of therapy.

III. Chemotherapy

A. Chemotherapeutic Evaluation of Compounds Against Epithelial Tumors in BALB/c mice. (with M. Kende)

In the past year a new model consisting of the primary implantation of a non-producer clone of BALB/3T3 cells transformed *in vitro* by the Kirsten strain of murine sarcoma virus (KiMSV) was utilized to evaluate the effects of anticancer agents with diverse mechanisms of action. The chemotherapeutic response of the KiMSV transformed carcinoma is generally that of a slow growing tumor. It is susceptible to alkylating agents and to the non-cell-cycle-specific antimetabolite 5-FU. The tumor also responds to treatment with the S-phase specific agent methotrexate. It is of interest that adriamycin exhibited definitive activity in this system.

B. Chemotherapy of Primary Moloney Virus-Induced Leukemia in BALB/c Mice. (with M. Kende)

It was previously reported that dimethylbenzyl rifampicin increased the lifespan of mice with primary Moloney virus-induced leukemia. In a series of confirmatory experiments the results have been variable. Administration of dimethylbenzyl rifampicin by the oral route gave a 22% increase in the lifespan of the animals. The primary Moloney virus-induced leukemia would appear to be suitable for remission induction plus remission maintenance protocols. The combination of vincristine plus prednisone elicited a significant remission rate and palmo ara-C was effective in remission maintenance. It is possible to measure the Moloney leukemia virus titer in serum and an attempt was made to determine whether the virus titer would be an indicator of extent of disease. It was determined that on effective remission induction and maintenance there was no correlation since the virus titer did not change either with remission or during any of the maintenance regimens.

C. Chemotherapy of Leukemia P1798 with Vincristine Plus Cytosine Arabinoside. (with Dr. S. Vadlamudi)

It was indicated in previous studies that leukemia P1798 was a potential model for the study of remission induction and remission maintenance. A further study was conducted to investigate the effectiveness of cytosine arabinoside as a remission maintenance agent following remission induction with vincristine. It was found that although cytosine arabinoside was more effective than vincristine when the drugs were given individually, treatment with vincristine every 7 days starting from the 7th day following leukemic inoculation plus treatment with cytosine arabinoside daily from the 7th day yielded an improved therapeutic response. The results suggest the desirability of further investigation of lymphosarcoma P1798 as an experimental model for remission induction and remission maintenance studies.

D. Studies with the MOPP Regimen in Mice Bearing Leukemias L1210, P1798 and P388. (with S. Vadlamudi and R. K. Johnson)

Studies have continued in an attempt to find appropriate animal models for studying multiple drug combination chemotherapy. MOPP has been demonstrated by DeVita et al. to be effective clinically as a four-drug combinatin (nitrogen mustard, oncovin, procarbazine and prednisone) in the treatment of advanced Hodgkin's disease. MOPP has now been tested in leukemias L1210, P1798 and P388. In none of the three tumor systems tested was the MOPP protocol the most effective therapy. If indeed the MOPP protocol is more effective clinically than combinations of three or two drugs or the drugs employed individually none of three experimental tumor systems tested would appear to be suitable for testing such combinations.

E. Studies with Mitomycin C and a Derivative K-36. (A. Miyakawa and S. Vadlamudi)

It was previously shown that mitomycin C is highly active against a broad spectrum of animal tumors and that one of its derivatives K-36, although considerably less toxic, yielded an approximately equal increase in survival time in the leukemia L1210 system. It has now been demonstrated that with both mitomycin C and K-36 therapeutic synergism was observed when either drug was administered as a single dose and sequential therapy with daily administration of methotrexate was then applied. The data suggest that the sequential treatment with K-36 plus methotrexate may be more effective than treatment with mitomycin C followed by methotrexate.

IV. Immunochemotherapy

A. Immunochemotherapy of AKR Spontaneous Leukemia. (with M. Kende)

Evaluation of compounds for antileukemic activity was continued with single, combination, and remission induction plus remission maintenance protocols. Intermittent weekly administration (4 injections) of cyclophosphamide was highly effective for remission induction and in increasing the survival time of the AKR mice. Daily administration of palmo ara-C on a chronic schedule was also highly effective. The combination of prednisone plus thioguanine was highly effective for remission induction yielding a remission rate of 87-96%. It appeared to be more effective than prednisone plus vincristine or prednisone plus 6-mercaptopurine. Following remission induction with vincristine plus prednisone administration of glaxo freeze dried BCG vaccine increased the survival time to 85%. Similarly the Pasteur live vaccine increased the survival time to 67%. It is of interest that these vaccines exerted no effect where there was no remission induction.

B. AKR Leukemia as a Model for Immunosuppressive Studies of Antileukemic Agents. (with M. Kende)

A model using first passage AKR leukemia has been standardized for the evaluation of immunosuppressive properties of chemotherapeutic agents. In this system cellular immunosuppresion is indicated by an increase in the gamma globulin titer on injection of B_6AKF_1 spleen cells, while humoral immune suppression is shown by the absence or decrease of anti-SRBC. In this system the agent tested for immunosuppressive activity is injected three days prior to inoculation of the leukemic cells. The therapeutic activity of the test agent is determined by the administration one day after inoculation of the leukemic cells. Employing this system it was determined that cyclophosphamide (200 mg/kg) had a strong therapeutic effect which was not altered by the moderate immunosuppression with this dosage of drug. This system has potential for the evaluation of the immunosuppressive properties of agents that are active against spontaneous or first generation transplanted AKR leukemia.

C. Collateral Sensitivity and the Levels of Sialic Acid in Sublines of Leukemia L1210. (V. V. Subba Reddy and J. Choudhry)

Leukemic L1210 sublines resistant to ara-C and 6-mercaptopurine exhibit collateral sensitivity to BCNU. An increase in levels of sialic acid was observed in ara-C and 6-mercaptopurine L1210 resistant sublines. In vivo exposure to BCNU resulted in further increase in sialic acid in the sublines. In contrast cyclophosphamide treatment which did not elicit collateral sensitivity was devoid of any effect on the levels of sialic acid. In what way the alterations in levels of sialic acid may be related to the phenomenon of collateral sensitivity remains to be determined.

D. In Vitro and In Vivo Assays of Antiviral Compounds Against Moloney Sarcoma Virus. (M. Kende and D. Houchens)

Additional studies were conducted on the Moloney sarcoma virus-induced rhabdomycosarcoma. This primary virus-induced tumor system is of interest for a number of reasons. (1) The tumor is induced very rapidly in the appropriate host. (2) The local tumor can be accurately sized. (3) The tumor is highly antigenic so that immunostimulatory or depressive compounds can be assayed with the model. (4) The tumor can be excised permitting the combination modality surgery plus chemotherapy. (5) The etiologic agent can be quantitated in vivo and in vitro. (6) The tumor possesses reverse transcriptase, thus inhibitory compounds of that enzyme can be selected with it. (7) The tumor can be inhibited with antibody or interferon. In one study BALB/c mice were inoculated with 10^4 focus forming units (FFU) of MSV and diphenylhydrazone 3-formyl-rifamycin SV(DPHFR) treatment was initiated immediately and continued daily for 45 days. This treatment did not increase the median survival time nor change the percent of tumor incidence or the mean tumor size. However, when 10^4 FFU of MSV was incubated for 30 minutes at 37° with DPHFR, marked retardation of tumor growth and tumor size occurred. It would appear that DPHFR has potential prophylactic or even therapeutic value if intimate contact between the drug and the virus can be established.

The remainder of the major findings in collaborative program are summarized in the annual reports of the Tumor Biology and Immunochemotherapy Sections of the Laboratory of Experimental Chemotherapy and in the annual reports of Drug Research and Development Branches.

Proposed Course of the Project

The various aspects of the project will be continued and modified in accordance with laboratory findings and clinical interest.

Continued emphasis will be placed on developing the international program and world surveillance for drugs for screening, drugs of interest for the clinic and new modalities of therapy. Continued emphasis will be placed on activities related to the planning and coordination of the total DR&D research program and on the further development of the preclinical-clinical interrelationships with respect to the predictability value of animal test models, combination chemotherapy and combination modalities. It is planned to develop further the Laboratory of Experimental Chemotherapy and in the extension of its activities to involve it in collaborative investigations with the other segments of Drug Research and Development and the Division of Cancer Treatment as well as in collaborative research in the United States and abroad.

Honors and Awards

A. Goldin was elected as a member of the Council of the International Society of Chemotherapy.

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MAMMALIAN GENETICS AND ANIMAL PRODUCTION SECTION
OFFICE OF ASSOCIATE DIRECTOR FOR DR & D

INTRODUCTION

The Mammalian Genetics and Animal Production Section, during 1973, in general continued to function as it has in prior reporting periods. Providing healthy laboratory animals with well defined genetic characteristics has been its major goal. Supporting services included diagnostic laboratory projects and others associated with the continuity of the desired genetic characteristics of the animals and the murine tumors used for laboratory investigations. Concurrent activities provided guidance and counseling for problems of animal diseases and a variety of information to investigators in the biomedical scientific community.

Although the total number of animals furnished during 1973 decreased, the variety of animals increased. These changes were influenced by alterations in the testing and research programs of the several divisions in the NCI. Laboratory monitoring of the animal stocks was expanded in order to further improve animal quality.

The overall animal production methodology revolves around the major groupings of Primary Centers, Genetic-Production Centers, Rodent Production Centers and large-scale production colonies. Germfree systems are used to maintain insurance stocks of foundation colonies. Pedigreed starts are derived into isolators for propagation with defined microbial flora, and offspring are used as breeders in controlled environments. These facilities are maintained under rigid control measures, for animals produced are shipped for breeding purposes in large-scale production colonies.

Expansions of effort in the supporting services laboratories were of minor consequence due to funding limitations. It was possible, however, to implement refinements in techniques for the elucidation of the causes of several potential epizootics. Minor research projects involved the implications of the presence of several species of microflora upon the health of rodents and how they could effect the screening program. Other projects concerned the identification of karyotypes of several murine tumors, blood parameters of germfree inbred rats, the histology and physiology of the South American agouti, comparisons of viral and bacterial resistance and susceptibility in several inbred strains of mice, and the identification of normal intestinal flora of Chinese hamsters and Mongolian gerbils. Collateral activities were associated with the development of standards; improvements in protocols; conferences on the use of laboratory animals, design of facilities and laboratory animal medicine; and participation in National and International meetings.

The Section was requested to furnish the categories of animals listed in Table I. The production of several additional stocks and hybrid mice were acquired and/or developed during this period. This group consisted of mice homozygous and heterozygous for the athymic nude characteristics, and $AKD2F_1$ and $D2AKF_1$ hybrids. Breeding animals for the overall program originated from the various centers with some exceptions. The majority of the AKR's required for the production colonies were obtained from the Jackson Laboratory as well as a small percentage of the C57BL/6 and DBA/2 mice. The small number of beagle hounds, rabbits and guinea pigs used for toxicologic studies does not justify U.S. Government-supported breeding colonies. Consequently, vendors for these animals furnish the breeding stocks. The Section was also instrumental in furnishing tumored animals to research institutions other than those performing Drug Evaluation Studies under the terms of contracts with the NCI.

Reliance by DCCP upon the MG&APS for animals to be used in carcinogenic investigations diminished during this period. These species are being supplied to contractors from the Animal Farm of the FCRC, and they are also furnished to on-going projects at the Frederick facility.

The Section staffing consisted of one senior staff member, one professional associate, one animal contracts assistant, one clerk-typist, one animal caretaker supervisor, and four animal caretakers. The animal caretaker staff function in DR&D in-house laboratories.

The senior staff member and the professional associate served as Project Officers and/or Assistant Project Officers for the contracts listed below.

Primary Centers for Rodents	5
Genetic Production Centers	5
Rodent Production Centers	7
BALB/c Genetic Center	1
Inbred Mouse Production	1
$B6D2F_1$ Hybrid Mouse Production	11
$CD2F_1$ Hybrid Mouse Production	5
Beagle Hound Production	2
Disease Diagnosis and Research	3
Histocompatibility Studies	2
Testing and Research Laboratories	3
Standards for Care and Maintenance	1
Conferences on Laboratory Animal Medicine and the Use of Laboratory Animals	1

The senior staff member and professional associate served as ex-officio Assistant Project Officers for advice/guidance/counseling in animal husbandry practices for all contract-supported testing and research laboratories.

ACCOMPLISHMENTS

Animal Production

The total number of animals furnished during this period decreased in comparison to the preceding one by approximately 3.0%. The most significant reduction was in the area of outbred rats--approximately 64%--which had been furnished to contractors supported by DCCP. Requirements for outbred mice increased by approximately 30%. However, these mice represented only 0.9% of the total number of animals furnished, and the impact upon the program was negligible. The use rates of B6D2F$_1$ and CD2F$_1$ hybrids decreased by 3.7% and 16.1% respectively. The majority of all animals were furnished to DCT projects. Support was provided to DCCP, DCBD, and on-campus laboratories on an as-available basis. DCT/DCCP cost sharing for contracts was discontinued during this reporting period.

Through the years we have continued to rely upon the Jackson Laboratory for significant numbers of AKR, C57BL/6 and DBA/2 mice to serve as breeders in large-scale production colonies. Although dependence upon DBA/2 mice from this source for this purpose has been eliminated, we continue to purchase approximately 20% of the C57BL/6's and approximately 95% of the AKR's from the Jackson Laboratory.

Primary Genetic Centers

The two centers most recently activated, i.e., Leo Goodwin Institute for Cancer Research and Washington State University, attained the projected workscope levels. All isolators are fully populated and production colony cages have received the requisite number of breeding animals. Offspring from both centers were transferred to subsidiary centers as replacements and to start new colonies in bio-containment facilities.

The inbred rat center located in the Tulane School of Medicine derived two additional strains. Several small-scale production colonies were established in order to make available groups of rats unavailable from other sources for studies supported by DCT and DCBD. This project has been extremely successful in achieving its objectives, and it would be worthwhile to expand this effort. The Principal Investigator and his professional associates have published several reports based upon the use of germfree rats in comparison to observations performed with conventionally-reared counterparts.

The Primary Center maintained by the ARS/Sprague-Dawley Company continued to be sub-divided into four distinct tasks. This mechanism is useful for charting rates of progress for the segments and also provides a useful device for the identification and measurement of expenditures. Isolator contaminations have been nominal and have not created a noticeable effect upon the overall program. Safeguards for such occurrences are built into the system by redundancy of counterpart colonies in two or more isolators. Several strains were derived in order to furnish clean breeding stocks for other centers. Efforts to develop germfree hamsters have been unsuccessful. However, a modest level of the task workscope will be continued in view of the fact that other workers have been equally unsuccessful, and the objective is worthwhile.

The Charles River Breeding Laboratories project encompasses four tasks. Task 1 includes isolators for the derivation and maintenance of stocks and strains of rodents. During this reporting period a colony of athymic nude mice was installed in isolators. This arrangement created an ideal environment, avoiding the necessity to offer antibiotics in order to maintain these mice in good health. Reproduction has been excellent, and substantial numbers of animals have been produced. Many of the bio-containment spaces are located in portable buildings, affording separation of species and strains and avoiding intercurrent infections. Task 2, located in Calco, Italy achieved full production status during this period. At this point, the Calco facility is furnishing approximately 80% of the animal requirements for the DCT projects with the Mario Negri Institute in Milan, and the Jules Bordet Institute in Brussels. Tasks 3 and 4 continued to demonstrate excellent rates of progress and have maintained pace with the workscope objectives without difficulty.

The Primary Centers have become an important adjunct to the animal provision program and are the basis for the production of breeding animals. Even though the possibility of an epizootic(s) will always exist, the effects will be minimal because sources for clean restarts are available. The construction of new plants with controlled environments by contractors should be supported by the availability of clean breeding stock with defined microbial flora.

Genetic-Production Centers

These centers furnished pedigreed and production colony animals in substantial numbers. The program to replace conventional stocks with derived animals from Primary Centers was accelerated during this period. The Texas Inbred Miceo Co. constructed a new building in accordance with a maximal control concept in order to accommodate derived populations. Several contractors have improved their facilities and strengthened husbandry practices. The systems in effect in all Genetic-Production Centers exceed all minimal standards promulgated for the care and maintenance of laboratory animals.

Epizootics have been non-existent. Minor problems which appeared were quickly diagnosed and resolved without difficulty.

Rodent Production Centers

These resources function as producers of animals for special laboratory programs, as ancillary sites for the production of breeding animals, and to supplement supplies of animals in correlation with changes in laboratory studies. The major portion of their current efforts are assigned for the production of weanling and aged AKR's. These centers also supply a significant portion of the animal requirements for tumor systems other than the L1210. Several of the current contractors have constructed new facilities with programmed climatic controls for these purposes. The results have been excellent, as was evidenced by the quality of animals produced. These centers could be expanded if it is determined that a reduction of support from the Jackson Laboratory is considered desirable.

Hybrid Mouse Production

$B6D2F_1$ and $CD2F_1$ production was reduced as noted above, due to the use of the "mini-screen" for compound evaluations. This reduction reflected an average effect because the hybrids were in short supply early in the year and excesses developed during the closing months of the year. The shortages were in large measure due to the phasing-in of several new contractors, one of whom continued to experience production problems. A management change served to correct a few of the deficiencies.

In-House Animal Colony Activities

The provision of animals for DR&D in-house research was confined to the numbers which could be humanely and properly cared for by the limited caretaker staff assigned to us for these purposes. This staff is responsible for the conditioning of animals before they are used for experimentation, and the care of research animals. Special breeding projects formerly conducted by this group were discontinued due to lack of sufficient time to properly perform these tasks.

Service Laboratories

The disease diagnostic laboratories are primarily concerned with the routine screening of excreta and body fluids in order to identify the presence or absence of bacteria and viruses which may be potential causes of diseases and/or interfere with laboratory investigations. Although these studies focus upon Salmonella spp., Pseudomonads, and a group of fifteen murine viruses, unusual conditions receive detailed studies. As warranted, this laboratory performs investigations involving the precise identification of all intestinal flora, histopathology, parasitologic investigations, and studies to determine the presence or absence of Fungi imperfecti.

Epizootics were non-existent in colonies sponsored by this Section. The incidence of Salmonella spp. in contract colonies continues to remain at 0% level. Salmonella spp. levels in accredited but non-contract colonies were less than 0.5%. Positives were generally confined to one or two organizations, and the species identified were atypical, such as Blockley and Miamia. Pseudomonas aeruginosa positives are detected more frequently and, when brought to the attention of colony managers, were usually corrected by means of increased chlorination and/or hydrochloric acid in water at pH 2.5. The availability of two bacteriology laboratories affords opportunities for processing sufficient samples and performing confirmatory studies. These investigations were performed by the IIT Research Institute and the Battelle Memorial Institute.

There was positive evidence that epizootics of Infectious ectromelia did exist. The fact that requests for vaccine were received from abroad as well as from institutions in the United States represents preventive practices and corrective measures.

The profile of viruses in the current diagnostic scheme appears adequate for the screening program. They include those most frequently implicated in rodent epizootics, LCM which is a human as well as an animal pathogen, and others of research interest. It is questionable whether it would be worthwhile to include additional viruses. The mere fact that they are present may create an aura of apprehension even though they may not exert a measurable effect upon the outcome of laboratory investigations. This philosophy does not necessarily imply that no further consideration will be given to the inclusion of additional viral agents. For example, LDH virus was recently included for diagnostic studies involving the tumor bank. Animal populations and tumors could be tested more frequently if another virus laboratory was available. A second laboratory would also be useful for confirmatory testing, particularly if a viral agent potentially pathogenic for humans was reported by the first laboratory. Virus studies were performed by the College of Medicine and Dentistry of New Jersey.

The contract with Health Research, Inc., for histocompatibility studies provided requisite assurance that mouse sublines were interchangeable and equally useful for a particular study. All strains readily retained skin grafts reciprocally with counterparts from the NIH colonies. During this period, this contractor also characterized the standard karyotype of the Lewis lung carcinoma.

The Mason Research Institute continued to perform genetic monitoring of inbred rat strains by means of skin grafts and the identification of the Hag antigens.

The incidence of dead and ill animals reported by consignees during 1973 was 2.851%, a decrease of .065% compared to the preceding year. Significant reductions in morbidity and mortality figures below this rate will be difficult to achieve unless programs are developed for periodic decontamination of laboratory areas. Evidence is accumulating that super clean animals eventually succumb to infections in heavily contaminated laboratories. This is particularly true when these animals are used for chronic studies. Filter covers on cages are a solution for this problem. Even though they are being used for many carcinogenic studies, many other areas do not employ this technique.

The Athymic Nude Mouse

A start in these mutant mice was received from Denmark. A few were used as a nucleus for an isolator colony and the balance were bred in a semi-controlled environment. Subsequently, large-scale production was undertaken in several Rodent Production Centers. Offspring have been transferred to the BCRC, several contract laboratories, and several on-campus laboratories. Minor problems have been resolved, and the current weekly production ranges between 200-300 per week. Since the production colonies have been completely populated, substantial numbers of the animals will be available within a few weeks.

The gene has been put on the BALB/cAn and, at this time, eight backcrosses have been completed. This genetic trait will be put on C57BL/6, DBA/2 and SJL/J backgrounds during the immediate future.

The Accreditation Program

The DR&D Accreditation Program continues to be a prime determinant for the evaluation of animal production organizations as potential sites for production contracts. Accreditation status is one of the qualifications for organizations who request copies for an RFP. Although accreditation is accorded for a period of one year, a minimum of two inspection visits per year are made by MG&APS staff.

Due to understaffing during 1973, visits to non-contract organizations were curtailed. The number of site visits made during this period are listed below.

Primary Genetic Centers	20
Genetic-Production Centers	20
Rodent Production Centers	21
Inbred Mouse Production Colonies	2
Commercial Rodent Production Colonies	15
Hybrid Mouse Production Colonies	22
Research Animal Colonies	15
Service Laboratories	10
Miscellaneous Visits	35
	160

Standards, Conferences, Meetings

Task Order 6 with the National Academy of Sciences provided for the development and publication of the following reports:

Recommendations to Editors of Scientific Journals for a Standardized Minimum Description of Animal Treatment.

Standards and Guidelines for Defined Rodents and Rabbits.

Standards and Guidelines for Defined Wild Caught Old World Monkeys.

Standards and Guidelines for the Breeding and Management of Laboratory Animals: Dogs.

Standards and Guidelines for Non-Human Primates, 2nd edition.

ILAR Brochure.

Information accumulated in this manner portrays not only current advancements in this field, but also serves as a stimulus for the development of additional improvement in the humane care and treatment of laboratory animals.

Task Order 16 with the National Academy of Sciences sponsored the following conferences during 1973:

Animal Models and Genetic Stocks Information Exchange.

Animal Models for Biomedical Research: Invertebrates.

International Symposium on Germfree Research.

This Task Order provides a forum for discussions of mutual problems in [illegible] care and use and Laboratory Animal Medicine, and the opportunity to develop solutions. These conferences also provide a means for interchanges of information, particularly in the area of suitable animal models and animals with naturally occurring counterparts of human abnormal conditions.

ILAR standards and conferences planned for the future:

Animal Models of Metabolic Diseases.

Environmental Factors Influencing Biomedical Research.

Future Use of Animal Models and Systems in Biomedical Research.

Nomenclature of Invertebrates.

Workshop on Animal Models for Blood Diseases.

Development of Standards for the Hypertensive Rat.

Development of Standards for the Athymic Nude Mouse.

Guide to the Care and Use of Marine Invetebrates.

Technical Assistance

The Section responded to an increased number of requests for information, guidance and counseling for the alleviation of animal diseases, methodology in animal husbandry practices, building design, sources for animals and other biological materials, program planning, training programs, and animal care equipment design.

The MG&APS and the Chemotherapy exhibits were presented by invitation during the annual meetings of the American Veterinary Medical Association, the American Society for Microbiology, and the American Association for Laboratory Animal Science.

Proposed Course of Project

Efforts will be continued to replace conventionally reared laboratory animal stocks with counterparts produced in controlled environments. The rapidity of conversions will be dependent upon expansions of isolator systems and bio-containment facilities and, of course, the availability of funds. Sufficient numbers of animals became available from Primary Centers during this period for valid evaluations of their usefulness for quality improvement in the various stocks. The results were most gratifying and provided sufficient proof that the system should be applied to the entire animal provision program.

There is a requirement for in-house small-scale investigations into diagnosis, treatment, causes and prevention of laboratory animal diseases. Information acquired by means of such pilot studies could be applied to the production colonies and research animal colonies. Implementation of these projects will be dependent upon Section staffing possibilities.

Investigations in the field will include the evaluation of "clean" animals for program studies; physiologic and blood parameters of isolator animals and others with defined microbial flora; tumor growth characteristics and the effects of compounds upon defined animals; the effects of variations in intestinal flora upon the health of laboratory animals and their responses to laboratory procedures; and the durability of defined animals used for chronic studies in conventional laboratory environments. The surgical/isolator program for the derivation of conventional stocks and strains will be continued.

Publications

1. Poiley, S. M., Brant, H. G., Dorey, J. L., Kalbach, H., Otis, A., Reeder, C. R., Van Hoosier, L. G., Jr., and Wheadon, J.: Procurement Specifications. IX. Defined Laboratory Rodents and Rabbits. I.L.A.R., NAS/NAE, NRC, 1973.

2. Poiley, S. M.: Observations on the reproductive performance and laboratory uses of inbred mice whose ancestors were derived via surgical/isolator techniques. In Heniger, J. B. (Ed.): Germfree Research, Biological Effect of Gnotobiotic Environments. New York, London, Academic Press, 1973, pp. 627-634.

3. Schiff, L. J., Barbera, P. W., Shefner, A. M., Poiley, S. M.: Stability of resistance-susceptibility characteristics in two inbred mouse strains. Lab. Anim. Sci. 23:522-524, 1973.

4. Schiff, L. J., Barbera, P. W., Shefner, A. M., Poiley, S. M.: Microbial flora and viral contact status of Chinese hamsters (Cricetus griseus). Lab. Anim. Sci. 23:899-902, 1973.

5. Poiley, S. M., Ovejera, A. A., Otis, A. P., and Reeder, C. R.: Reproductive behavior of athymic nude (nu/nu BALB/cA/BomCr) mice in a variety of environments. Proceedings of the 1st International Workshop on Nude Mice, Scanticon, Aarhus, Denmark. Gustav Fischer Verlag-Stuttgard, 1974. In press.

6. Poiley, S. M.: An evaluation of requirements for defined animals in biomedical research. In Advances in Pharmacology and Chemotherapy, Vol. 12, Academic Press, New York, 1974. In press.

TABLE I

ANIMALS SUPPLIED DURING CALENDAR YEARS 1972 AND 1973

Inbred Mice	1973	1972	+ Increase - Decrease	
A/HeCr	17,381	14,359	+	3,022
A/JCr	2,635	2,135	+	500
A/JCr litters	3	--	+	3
AKR/Cr	207,726	249,637	-	41,911
AKR/Cr pregnants	30	10	+	20
AKR/Cr aged	110,922	114,558	-	3,636
A.θ-AKR	839	--	+	839
AL/NCr	1,231	1,918	-	687
BALB/cCr	264,299	216,015	+	48,284
BALB/cCr pregnants	5,745	7,206	-	1,461
BALB/c litters	678	348	+	330
BALB/cJCr	673	676	-	3
BALB/cfC3H/HeCr	42	--	+	42
BALB/cfC3H/HeCr pregnants	105	--	+	105
B10.A/Cr	1,585	3,975	-	2,390
B10.A(2R)/Cr	480	642	-	162
B10.A(5R)/Cr	5,805	6,569	-	764
B10.A(5R)/Cr pregnants	2	--	+	2
B10.A(5R)/Cr litters	3	--	+	3
B10pd/CzCr	3,374	1,847	+	1,527
B10.129(5M)/Cr	1,622	10	+	1,612
BRVR/SrCr	804	288	+	516
BRVR/SrCr litters	--	5	-	5
BRVS/SrCr	1,565	926	+	639
BSVR/SrCr	292	287	+	5
BSVR/SrCr litters	--	4	-	4
BSVS/SrCr	2,419	959	+	1,460
BSVS/SrCr	2	--	+	2
CBA/JCr	2,757	3,390	-	633
CBA/JCr litters	2	--	+	2
C57BL/6Cr	241,573	207,038	+	34,535
C57BL/6Cr pregnants	47	136	-	89
C57BL/6Cr litters	5	--	+	5
C57BL/10ScCr	4,043	3,673	+	370
C57BL/10ScCr litters	2	--	+	2
C57BL/KaCr	1,614	2,166	-	552
C58J/Cr	431	302	+	129
C57L/Cr	5,301	2,052	+	3,259
C57L/Cr pregnants	--	34	-	34
CFW/PlCr	1,362	1,496	-	134
C3H/BiCr pregnants	65	--	+	65
C3H/HeCr	131,620	83,233	+	48,387
C3H/HeCr pregnants	349	67	+	282
C3H/HeCr aged	5,384	1,753	+	3,631

TABLE I
(Continued)

Inbred Mice	1973	1972	+ Increase - Decrease
C3Hf/HeCr	9,705	6,737	+ 2,968
C3Hf/HeCr pregnants	52	5	+ 47
DBA/2Cr	140,036	113,979	+ 26,057
I/Cr	65	18	+ 47
NIH/PlCr	1,738	2,906	- 1,168
NIH/PlCr pregnants	4	18	- 14
NIH/PlCr litters	3	1	+ 2
NZB/Cr	3,002	2,518	+ 484
NZB/Cr pregnants	3	--	+ 3
NZB/Cr litters	2	1	+ 1
NZW/Cr	841	1,822	- 981
NZW/Cr pregnants	1	--	+ 1
NZW/Cr litters	--	1	- 1
P/JCr	189	37	+ 152
PRI/PlCr	1,908	2,318	- 410
PRI/PlCr litters	--	2	- 2
SJL/JCr	5,338	5,847	- 509
SM/JCr	1,808	2,086	- 278
STR/Cr	10	--	+ 10
	1,182,419	1,058,172	+ 124,247
Pregnants	6,403	7,476	- 1,073
Litters	700	362	+ 338
Hybrid Mice			
AKD2F_1	1,023	959	+ 64
B6AF_1	3,162	3,534	- 372
B6AKF_1	5,696	7,686	- 1,990
B6AKF_1 pregnants	--	6	- 6
B6C3F_1	55,553	53,562	+ 1,991
B6C3F_1 pregnants	--	6	- 6
B6C58F_1	--	91	- 91
B6D2F_1	3,102,160	3,221,422	- 119,262
BkC3fF_1	--	1,751	- 1,751
CB6F_1	7,404	8,150	- 746
CB6F_1 pregnants	27	12	+ 15
CD2F_1	609,790	727,207	- 117,417
C58B6F_1	--	254	- 254
C3fC3F_1 pregnants	295	9	+ 286
C/StD2F_1	540	210	+ 330
C3C/StF_1	421	132	+ 289
C3PF_1	636	339	+ 297
D2AKF_1	1,855	1,572	+ 283
D2C/StF_1	215	83	+ 132
D2PF_1	505	166	+ 339
PD2F_1	643	170	+ 473

TABLE I
(Continued)

Hybrid Mice	1973	1972	+ Increase - Decrease
ZWZBF$_1$	1,460	1,518	- 58
ZWZBF$_1$ pregnants	146	159	- 13
	3,791,063	4,028,806	- 237,743
Pregnants	468	192	+ 276
Outbred Mice			
Crl:(ICR)	8,900	2,400	+ 6,500
Cr:MGAPS(S)	22,328	15,144	+ 7,184
Cr:MGAPS(S) pregnants	268	13	+ 255
Cr:MGAPS(SW)	14,284	14,667	- 383
Nu/nu	199	--	+ 199
	45,711	32,211	+ 13,500
Pregnants	268	13	+ 255
Inbred Rats			
ACI/Cr	1,211	2,022	- 811
ACP/Cr	192	192	0
ALB/Cr	269	331	- 62
AUG 2991/Cr	1,243	1,004	+ 239
AUG 990/Cr	96	--	+ 96
BD I/Cr	18	--	+ 18
BD II/Cr	67	--	+ 67
BD III/Cr	224	--	+ 224
BD IV/Cr	132	--	+ 132
BD V/Cr	9	--	+ 9
BD VI/Cr	155	--	+ 155
BD VII/Cr	44	--	+ 44
BD VIII/Cr	214	--	+ 214
BD IX/Cr	97	--	+ 97
BD X/Cr	195	--	+ 195
BN/Cr	22	193	- 171
BUF/Cr	2,004	2,063	- 59
BUF/Cr pregnants	--	4	- 4
COP 2331/Cr	1,749	1,550	+ 199
COP 2331/Cr litters	--	1	- 1
F344/Cr	77,916	93,547	- 15,631
F344/Cr pregnants	98	107	- 9
F344/Cr litters	--	1	- 1
LE/Cr	354	193	+ 161
M520/Cr	990	1,011	- 21
NBR/PlCr	3,199	5,283	- 2,084
NBR/PlCr litters	2	--	+ 2
OM/Cr	216	324	- 108
SD/MCr	2,308	2,909	- 601
SH/Cr	970	1,116	- 146

TABLE I
(Continued)

Inbred Rats	1973	1972	+ Increase - Decrease
SH/Cr litters	3	--	+ 3
S5B/PlCr	359	450	- 91
W/FuCr	8,299	5,263	+ 3,036
W/FuCr pregnants	8	10	- 2
W/LCr	8,107	5,266	+ 2,841
W/LCr litters	6	--	+ 6
YOS/Cr	2,567	2,541	+ 26
ZIM/Cr	520	19	+ 501
	113,746	125,277	- 11,531
Pregnants	106	121	- 15
Litters	11	2	+ 9
Hybrid Rats			
$F344BNF_1$	--	1	- 1
$WFBNF_1$	301	348	- 47
	301	349	- 48
Outbred Rats			
Cr:MGAPS(Gunn)	256	183	+ 73
Cr:MGAPS(OM)	8,129	38,711	- 30,582
Cr:MGAPS(OM) pregnants	--	24	- 24
Cr:RAR(SD)	12,090	18,091	- 6,001
Cr:RAR(SD) pregnants	243	180	+ 63
	20,475	56,985	- 36,510
Pregnants	243	204	+ 39
Hamsters			
Cr:RGH(SYR)	46,050	56,328	- 10,278
Cr:RGH(SYR) pregnants	1,001	964	+ 37
Beagles	328	600	- 272
Gerbils	106	212	- 106
Guinea Pigs			
Strain 2	52	86	- 34
Strain 13	2	49	- 47
	54	135	- 81

TABLE I
(Continued)

GRAND TOTALS	1973	1972	+ Increase - Decrease
Inbred Mice	1,182,419	1,058,172	+ 124,247
Pregnants	6,403	7,476	- 1,073
Litters	700	362	+ 338
Hybrid Mice	3,791,063	4,028,806	- 237,743
Pregnants	468	192	+ 276
Outbred Mice	45,711	32,211	+ 13,500
Pregnants	268	13	+ 255
Inbred Rats	113,746	125,277	- 11,531
Pregnants	106	121	- 15
Litters	11	2	+ 9
Hybrid Rats	301	348	- 47
Litters	--	1	- 1
Outbred Rats	20,475	56,985	- 36,510
Pregnants	243	204	+ 39
Hamsters	46,050	56,328	- 10,278
Pregnants	1,001	964	+ 37
Beagles	328	600	- 272
Gerbils	106	212	- 106
Guinea Pigs	54	135	- 81
	5,200,253	5,359,074	- 158,821
Pregnants	8,489	8,970	- 481
Litters	711	365	+ 346

PUBLICATIONS SECTION

Drug Research and Development

The Publications Section directs the preparation of Cancer Chemotherapy Reports (CCR), the principal scientific journal developed under the DCT Program, officially clears all DCT contract manuscripts, and provides editorial assistance to the DCT staff on a time-available basis. Until 1968, CCR was a single journal but in that year it was expanded to 3 parts. From its inception, CCR (and CCR Part 1) has published original contributions in clinical and experimental studies of cancer chemotherapy, including complete works, preliminary investigations, commentaries and reviews, symposia, and letters pertinent to published articles. All candidate manuscripts are reviewed by 2 or 3 investigators familiar with the subject matter and the final determination of acceptance or rejection is made by the Scientific Editors. CCR Part 1 is now in its 16th year of continuous publication with a total to date of 101 issues. In 1973, the dates of the last two issues were advanced to insure that the date of receipt by subscribers and the date of the issue were the same. For this reason only 4 issues appeared in 1973 (total of 524 pp). The journal is now being published on schedule for the first time since 1964. Six issues are planned for 1974.

CCR Part 2, which contains extensive and usually tabular studies on chemotherapy screening, structure-activity relationships, and other related subjects, has been published since 1968 as manuscripts and personnel have been available. In 1974, the 5th, 6th, and 7th issues (Vol. 4) have been or will be published (total of 692 pp).

There have been 7 issues to date of CCR Part 3, which contains non-reviewable program information. In 1973, 3 issues of Part 3 were produced (total of 576 pp): the proceedings of the 1st International Workshop for Therapy of Lung Cancer; an issue containing clinical brochures, toxicology reports, and the Protocols of the Laboratory of Toxicology; and the proceedings of the 3rd Joint Working Conference, DCT, NCI. One issue of Part 3 is planned for 1974.

In 1973, a new section entitled Brief Reports and Preliminary Communications was introduced in Part 1. Authors were invited to submit short clinical notes or the abbreviated results of preliminary work to this section. These reports have been processed through the editorial channels more quickly than a full-length manuscript, thus allowing an early publication date. The response to this new section has been excellent during the past year. Each issue has contained 3-8 Brief Reports and an increasing number are being submitted.

In 1974, a new section containing invited editorials was initiated; we plan to include one editorial in each issue of Part 1. A well-known scientist or physician is invited by a member of the Editorial Board to write briefly on a subject of current interest in cancer chemotherapy. We hope this section will encourage more interest in current topics, particularly through the submission of Letters to the Editor.

During the past 2 years, CCR has been approached more frequently to publish the proceedings of scientific meetings. In 1974, 4 issues will contain the results of scientific symposia, only 1 of which was sponsored by the NCI. We have found that these issues are always very well received based on the requests from subscribers for additional copies.

Comparative data on the CCR series of publications are shown in Table 1. The manuscripts accepted are reported monthly to the Editorial Office of the Journal of the National Cancer Institute, which in turn distributes this information in their monthly reports. The manuscripts received are reported monthly to the Associate Director, DR&D, and the Director, DCT.

More manuscripts were received in 1973 for all 3 parts of CCR than in any previous year. In addition, at the time of this writing (April 1974) we have received twice as many manuscripts as had been received at this time last year. We hope this increased interest in CCR is a result of a more careful selection of material to be published and a more timely publication of the accepted material.

Over the past 5 years publishing costs in this country have increased tremendously. This has naturally been reflected in higher operation costs for the Publications Section. However, in 1971 the average cost per page was $54.00 as compared to $51.00 in 1972.* Although this is only a slight decrease, it is important in view of the increased cost of services that is occurring nationwide. We have done several things to help bring about this drop: (a) As often as possible we supply our contract printer with camera-ready art prepared by this office rather than pay for his time to produce such art; (b) we now make many corrections on the finished reproduction proofs instead of requesting the printer to make such changes; and (c) the new Brief Reports are, by design, short and uncomplicated and therefore require less work by the typesetter.

Since 1967 CCR has been listed in Current Contents, Life Sciences. In February 1973, CCR was asked to be included in a new publication of current titles in collaboration with Science, Engineering, Medical and Business Data Ltd., Oxford, England, and in 1974, we were asked to establish a similar policy with the Japan Medical Service, which publishes a supplement to its Index of Japanese Medical Periodicals listing foreign publications. It is expected that this additional coverage of the material presented in the journal will increase the demand for subscriptions, particularly in other countries.

In 1964, CCR began sending corrected page proof to the Chemical Abstracts Service for abstracting and indexing of the chemical information. During FY 73 CCR was asked to establish a similar policy with the Biosciences Information Service (Biosis) in Philadelphia, and with Infordata International in Chicago, which publishes the Index to the Periodicals of the US Government. This trend toward more extensive coverage of CCR indicates a growing interest in the field and in CCR in particular.

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*These are the most recent years for which actual printing costs are available.

The Publications Section is also responsible for maintaining the free mailing list for CCR. CCR is available without cost to qualified medical groups, physicians, and libraries, and is for sale to others by the Superintendent of Documents, Government Printing Office. The approximate distribution, which varies with each issue, is as follows:

	3700	Foreign and domestic free mailing (includes ca. 175 at NIH plus storage copies for Section)
	1200	Superintendent of Documents (for paid subscriptions)
	367	Superintendent of Documents (for Depository Libraries)
Total	5267	

In the past year the number of paid subscriptions has increased from 500 to 1200. This figure will undoubtedly continue to increase because of a recent limit (3300) established by the OMB on the number of free subscriptions.

Problems with the service rendered by the GPO in maintaining the mailing list have brought about a change in the distribution procedures. Beginning in 1973 the mailing list was transferred to the Distribution Section, Printing and Reproduction Branch, ODA, NIH. This conversion has allowed more rapid service in 2 areas: (a) adding, deleting, and changing names on the list, and (b) obtaining mailing tapes to be used by the contract printer in mailing the issues to the subscribers. NIH has been able to perform these functions in one fourth of the time GPO previously required.

Another function of the Section is the clearance of DCT contract manuscripts for publication. In 1973, 149 of these manuscripts were cleared, and during the first quarter of 1974, 54 manuscripts were cleared. In assuming the responsibility for clearance of these manuscripts, the Publications Section checks all NSC numbers mentioned in the papers and notifies the DDB when compounds must have publication clearance from suppliers. The authors are instructed to reword footnotes as necessary to give proper credit to the DCT. The Section ensures that the Project Officer sees all papers and signs the correct forms. Records and reprints are kept for all manuscripts cleared, and quarterly reports are prepared for distribution to staff personnel.

Table 1.--Summary data on the CCR Publications Series

	CY 70	CY 71	CY 72	CY 73	CY 74 (Jan 1-Apr 5)
Mss. received	122 (pt 1) 5 (pt 2) 1 (pt 3)	107 (pt 1) 6 (pt 2) 11 (pt 3)	122 (pt 1) 4 (pt 2) 47 (pt 3)	148 (pt 1) 19 (pt 2) 77 (pt 3)	69 (pt 1) 1 (pt 2) 2 (pt 3)
Mss. accepted	68 (pt 1) 4 (pt 2)	73 (pt 1) 5 (pt 2) 11 (pt 3)	76 (pt 1) 5 (pt 2) 47 (pt 3)	112 (pt 1) 19 (pt 2) 76 (pt 3)	37 (pt 1) -- (pt 2) 2 (pt 3)
Issues published	6 (pt 1)	5 (pt 1) 1 (pt 2) 1 (pt 3) 7	6 (pt 1) 1 (pt 2) 2 (pt 3) 9	4 (pt 1) - 3 (pt 3) 7	Pt 1:1 (2 in press) Pt 2:1 (1 in press) (10 issues planned)
Pages published	492 (pt 1)	621 (pt 1) 246 (pt 2) 104 (pt 3) 971	814 (pt 1) 353 (pt 2) 181 (pt 3) 1348	524 (pt 1) - 576 (pt 3) 1100	Pt 1: 123 (320 in press) Pt 2: 180 (362 in press) (1700 pp planned)
Reviewers	140	164	197	167	88
Printing costs[*]	$15,334 (31.16 pg)	$33,284 (pt 1) (53.59/pg) $14,828 (pt 2) (60.27/pg) $4,564 (pt 3) (43.88/pg)	$37,715 (pt 1) (46.33/pg) $21,064 (pt 2) (59.66/pg) $10,863 (pt 3) (60.01/pg)	$28,000[+] (pt 1) $35,000[+] (pt 3)	Not available

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[*] Costs of GPO contract with Publications Press, Inc., Baltimore, Md.

[+] Estimated costs; actual figures not available April 5, 1974.

ANNUAL REPORT

DRUG DEVELOPMENT BRANCH

DRUG RESEARCH AND DEVELOPMENT

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

As the national and international effort on the eradication of cancer is accelerated, the expanded research, development and application will result. The major avenues presently being followed are radiation, surgery and chemotherapy, and to a lesser extent, immunotherapy. The two older techniques, radiation and surgery, are applied generally to the localized disease. The newer approach chemotherapy, has its strategy directed towards both the localized and the disseminated cancer. The foundation of chemotherapy is the drug itself. Consequently, the search for more effective less toxic, and broader acting agents will continue.

Major Branch Highlights

In addition to the Branch accomplishments listed later in the report, the major highlights are listed here:

1. A new methodology has been developed for the preparation of plant extracts for bioassay, consisting of an ethanol extraction followed by a preliminary fractionation. This has resulted in the removal of much extraneous material, the concentration of activity into two fractions and the production of several times as many active extracts as are being obtained by the old method.

2. Plant collecting has been made more selective following a program review which has identified the more promising families in which collection will be emphasized and the less promising families in which collection will be greatly reduced or dropped.

3. The number of new materials entering the program was doubled (51,334) for an all time high which resulted from a concentrated collecting effort in the U.S.

4. The successful chemical coupling of daunosamine to daunomycinone to provide the antibiotic daunomycin. This completes the second major phase of the synthesis of daunomycin or adriamycin. The practical synthesis of daunosamine was completed last year; only the completion of the adriamycinone remains.

5. Two hundred and sixty-two new suppliers of materials were added during the year.

6. A major symposium was held on structure-activity methodologies.

7. The number of clinical dose formulations have doubled over last year.

Objectives

The Drug Development Branch is engaged in a cooperative approach to the problems of cancer chemotherapy through the research and development of more effective chemical agents for use in humans. Past experience has clearly demonstrated that effective antitumor agents are not restricted to a single, or even a few, chemical classes but that the chemical-structural parameters are broad. One must conclude then that all effective agents are not operating by a single mechanism. Therefore, efforts are directed towards the chemical modifications of existing drugs to increase their activity and/or decrease their toxicity, and to search for new agents via chemical synthesis, or from natural sources. The latter category includes higher plants, marine invertebrates, insects, other animals, algae and fermentation filtrates. Materials from these sources will be evaluated in the primary screen and those agents demonstrating sufficient activity will be subjected to toxicolgical studies and possible clinical evaluation.

Research efforts are, in large measure, based on leads provided by the primary screens, biochemical and toxicological studies, pharmacokinetics observations, literature reports, and the surveillance of the worldwide scientific community for information on new drugs. As the vast storehouse of information accumulates and greater achievement is being experienced in the control of the more rapidly proliferating phase of cell growth or young tumors, the search for new structures aimed against the slower growing tumors as in specific areas, i.e. lung, breast, kidney, colon, central nervous system, etc. will be increased.

As part of the broad Division of Cancer Treatment Program, the Drug Development Branch has three major objectives:

I. Fundamental Chemical Research

   a. chemical synthesis of new compounds;

   b. extraction, isolation and identification of new active agents from natural sources;

   c. theoretical design of new chemical structures via structure-activity evaluations in collaboration with investigators of biochemistry, biology, toxicology, pharmacokinetics, and clinical trials; and

   d. development of methodology for drug assay.

II. Worldwide Surveillance

   a. information on potential antitumor chemicals and drugs; and

   b. discovery sources of new chemicals and drugs for biological evaluation.

III. Applied Services

a. accessioning and chemical nomenclature of materials entering the program;

b. development, maintenance and utilization of a computer data bank for chemical and drug information;

c. the correlation of chemical structure to biological activity;

d. chemical resynthesis and production of active agents;

e. Quality control and analytical evaluation of drugs and bulk chemicals for clinical and preclinical toxicological evaluations;

f. chemical synthesis of active radiolabeled drugs;

g. development and production of suitable dose formulations for use in clinical studies; and

h. storage and distribution of bulk chemicals and clinical dose formulations.

Implementation of Program

These objectives are carried out through the activities of the Branch's seven Sections which are:

I. Chemical and Drug Procurement Section: The objectives are divided into the synthesis, preparative, and quality control activities. The synthesis activities are involved with the synthesis of new compounds of diverse structures for antitumor evaluation. Thus far, drug treatment has had more success in fast growing on young tumors than in slow growing tumors. As the rapidly growing tumors become more and more under control, attention now will be refocused upon developing new drugs for the slower growing tumors, especially for colon, lung, breast, melanoma, etc.

The preparative activities include the process development and improvement of procedures for large scale production; the synthesis and isolation of large quantities of bulk chemicals and drugs for advanced screening, confirmational testing, pharmacological and toxicological evaluation, and clinical studies; and preparation of high purity radiolabeled materials for the chemotherapy studies. In addition, the procurement via purchase of large quantities of certain bulk chemicals and drugs needed by the Program are included in the preparative activiti

Chemical and quality control-analytical data are provided to the Food and Drug Administration (FDA) for the filing of Investigational New Drug (IND) applications.

II. Natural Products Section: The objective is the development of novel drugs from: 1) higher plants and animal (marine and insect) sources and 2) microbial sources. In support of these tasks, the Section maintains a worldwide program (partly contract and partly non-contract) for the collection of natural products, the preparation of extracts, fractionation of active extracts and the isolation and identification of the active components and in collaboration with the Chemical and Drug Procurement Section, provides for the acquisition of large quantities of active materials needed for toxicological and clinical studies. Also, the Natural Products Section directs a program for the investigation of three groups of organisms, the bacteria, the fungi, and the actinomycetes which have shown remarkable biosynthetic capabilities. This work requires development and chemical isolation. Surveillance of the literature is maintained as a means of obtaining new materials.

III. Drug Design and Chemistry Section: The objectives of the intramural laboratory effort are: (1) chemical research in the design and synthesis of new drugs for the treatment of cancer, (2) the chemistry of agents and drugs of interest in the treatment of cancer, (3) investigation of the chemistry of natural products as potential antitumor drugs, (4) research on the structure, purity, decomposition products and metabolites of antitumor drugs by analytical instrumental techniques, and (5) the development of structure-activity techniques to summarize existing data and to provide guidelines for analog synthesis.

Individual Progress Reports (Serial No. 3580, 3581) are presented later in this report.

IV. Clinical Drug Distribution Section: The objectives of this Section are to provide the Program with suitable formulated drugs for clinical evaluation. The principal activities of the Section include development of experimental dosage forms, production of various dosage forms for clinical investigative trial, surveillance of the quality of such dosage forms, procurement of commercially available pharmaceutical products, and storage and distribution of the clinical products used in the Cancer Treatment Program. In addition, the Section provides the necessary supportive data for the filing of IND applications with the F.D.A.

V. Chemical and Drug Records Section: The objectives are to 1) assist in the worldwide surveillance and collection of new drugs and provide the program with possible sources of such agents, 2) manage the acquisition of chemicals contributed to the Program, 3) develop, maintain, and utilize a chemical data bank on materials of use to the program, 4) develop structure-activity methodology for new materials to be synthesized in collaboration with the Chemical and Drug Procurement Section and the Drug Design and Chemistry Section, 5) maintain adequate storage and inventory of bulk chemicals, and 6) coordinate the distribution of materials to screeners and other investigators. Surveillance is also provided over industrial 'Commercial Discreet' products.

During this reporting period, the reorganization of the Drug Research and Development activity resulted in the transfer of the Data Processing and Computer Systems Development Section and the Scientific Records Section to the Drug Development Branch.

VI. Data Processing and Computer Systems Development Section: The objectives of the Section are to develop data processing systems to meet the scientific, operational control, and management planning needs of the Branch. The Section is primarily concerned with: (1) directing contract effort to implement a new automated Chemical Information System (CIS) which supports such activities as accessioning, storage, and distribution of compounds acquired for antitumor testing; (2) directing and coordinating the activities of the Contractor responsible for operating the Chemical Information Systems. In addition, the Section is engaged in pursuing a project to improve the utilization of automated literature and chemical files maintained by Federal agencies and industrial organizations to acquire compounds of interest to the program.

VII. Scientific Records Section: The objectives are to coordinate information on all new drugs acquired for testing in a manner that allows fast processing prior to biological evaluation and facilitates retrieval from the chemical file retrospectively by individual compound or by chemical class using manual, microfilm and computer system. In addition, the Section personnel (1) prepares or monitors the preparation of all chemical input in a standard format for duplicate checking, (2) maintains the master chemistry card files and suppliers addresses, (3) supervises the maintenance of computer name and formula indexes to the file, (4) prepares documents for the shipment of all new acquisitions, (5) provides retrospective searching of the file using the index and substructure search programs, and (6) investigates new methods to facilitate chemical information retrieval and dissemination.

Branch Staff

The Drug Development Branch is composed of a staff of forty persons consisting of seven senior professional scientists (GS-13 and above), eleven other professional staff members (GS-9 to GS-12), three PHS Commissioned Corps Officers, two Staff Fellows, four Visiting Fellows, and the remainder technical and clerical staff.

The Branch is divided into seven Sections and those responsible for each activity are as follows:

Drug Development Branch
Chief -- Dr. Harry B. Wood, Jr.

Chemical and Drug Procurement Section
Head -- Dr. Robert R. Engle

Natural Products Section
Head -- Dr. Jonthan L. Hartwell

Drug Design and Chemistry Section
Head -- Dr. John S. Driscoll

Clinical Drug Distribution Section
Head -- Mr. J. Paul Davignon

Chemical and Drug Records Section
Head -- Mr. Robert B. Ing

Data Processing and Computer Systems Development Section
Head -- Mr. Sidney Richman

Scientific Records Section
Head -- Mr. George F. Hazard

The Chemical Quality Control Unit (Head, Mr. C. A. Hewitt) operates as an activity under the Chemical and Drug Procurement Section. The Antibiotic Unit (Head, Dr. J. D. Douros) operates as an activity under the Natural Products Section.

Operation of Program

The methods for selecting new agents for input into the primary screen is gradually changing. More emphasis is being directed toward selectivity of agents and less emphasis towards random procurement. This should result in more active analogs of existing active materials, fewer biologic tests required, and logically, the number of active agents should be higher. Acquiring such preselected agents may be more costly. Changes in the screening protocol should be incorporated to include differentiation of analog activity, including toxicological evaluation where appropriate.

If one were to obtain a constant number of active materials, random screening would require a much larger input of compounds than a rational approach. Unfortunately, the state-of-the-art has not reached a degree of sophistication which allows a completely rational approach to drug design. Currently, (1973), we will acquire the largest number of compounds from random sources, but an increasing number of rationally designed compounds are being generated. While the rational method is preferred, the random approach will serve a useful purpose for the foreseeable future. Random input has thus far provided a number of active compounds and certainly the greatest number of unique structures. However, as preselection increases and as the structure-activity mosaic gradually takes shape, the choice of chemical compounds for introduction into the screen will emerge more from direct synthesis and will gradually replace random procurement. The gradual mutation from random input to directed selection of agents for screening will be generated from review and evaluation of chemical and biological data from within the program, in the literature and other related programs. Consideration is directed toward antitumor structure-activity relationships with emphasis on new and promising structural classes and de-emphasis or elimination of adequately studied classes or non-productive structural classes.

Analog synthesis will be aimed at improving those agents which have shown therapeutic effectiveness in the screen but have been abandoned later because of toxicological or other problems.

Synthetic analogs of known active drugs may possess superior properties because of better transport and greater resistance to degradation or greater binding to appropriate enzyme sites. Synthetic analogs also may produce longer or shorter duration of activity as the needs dictate, alter the solubility characteristics and improve the stability. Structural modification could also introduce new chromophore groups to aid in determining by analysis the drug level in the host. Analogs would also be used in mechanistic studies, and biochemical investigations which include areas of whole body drug distribution, drug metabolites, enzymatic activity, and the route and rate of excretion.

New chemical structures or analogs, developed through biochemical cell cycle kinetic studies, pharmacological and pharmaceutical rationales, and biological activities demonstrated in non-cancer programs, should provide additional input to the directed preselection and synthesis approach. Plans are underway to group together those agents which have shown no cross-resistance and determine if there are any structural similarities for future analog design. The same study will be made on those agents which have shown potentiations in classical biologic and/or biochemical screens for new drug synthesis.

In addition to the search for 'mitotically linked drugs', directed synthesis should also be aimed at affecting other phases of the cell cycle than the synthetic (S) phase or the mitosis (M) phase. The search for agents which have a high degree of organ localization will be undertaken and compounds containing this property should be considered as potential leads for chemical modification. Such specific localizing agents have been encountered with compounds containing the nitroso-moiety which may penetrate the blood-brain-barrier. A program has been developed to synthesize CNS active antitumor agents. Also, drugs localizing in the lung, kidney, colon, prostate, etc., will be considered in modifying their chemical structure with the objective of introducing antitumor properties. Organ specific screens are needed to evaluate such new types of agents. The increasing awareness that many antitumor drugs may possess latent carcinogenic properties will be considered in the hopes of eliminating such characteristics. Synthetic development may provide improved methods of synthesis which would achieve economic advantages in the production of large amounts of drugs for the clinic.

The Branch is continuing its search for natural products, which often provides an invaluable source of medicinal agents and new chemical structures. Plants continue to provide many extracts with significant tumor-inhibitory activity. It is important that the compounds responsible for this activity be isolated and characterized. Some will be useful in chemotherapy, others will be useful as tools in elucidating selective growth-regulating mechanism or as templates for syntheses of more effective agents.

Chemical modification and structural analogs of natural products will also provide unique compounds of interest. The continued follow-up of empirical observations and changes in the structure ofthe already effective agents will also add to our array of new material for investigations. The random but unique structures acquired from natural products--higher plants, fermentation products, algae, marine and insect products--will continue to be explored.

The Branch is keenly interested in new approaches to drug design. One such approach being exploited is the synthesis of analogs to develop and exploit structure-activity relationships. When a significant new activity has been established, there is need for a major effort to prepare and study related derivatives for the purpose of defining the relation of structure to biological activity. It is desirable to study a series of closely related active compounds to determine toxicity, transport, and other chemo-physical properties. Recognizing that such techniques are still in the early stages of development, effort will continue to improve and develop new methodologies. Several problems do still exist in this area of investigation; such as, obtaining the physical-chemical measurements and the fact that the physical-chemical measurements are quite exact whereas the biological testing data has a considerable amount of variation. It must be recognized that this approach may not necessarily give immediate answers since it may be necessary to develop new parameters. However, it is hoped that such studies will focus attention on synthetic areas of greatest potential and provide the chemist with more directed synthetic approaches. This is not to be interpreted that new structural classes wil be by-passed.

Symposia and Seminars Sponsored

In order to focus on various specific scientific areas of interest to the scientists of the Branch and DCT in general, the Drug Development Branch has sponsored the following symposia:

I. Structure-Activity Relationships
II. Quinones as Antitumor Agents
III. Synthesis of Adriamycin and its Analogs
IV. Tetrahydrouridine
V. Nucleosides

I. Structure-Activity

On March 4, 1974 the Drug Development Branch sponsored a symposium entitled 'Structure Activity Techniques in the Treatment of Antitumor Data', with 103 attendees. The purpose of this symposium was to enable NCI Staff and synthetic contractors to obtain an overview of the 'State of the Art' with regard to various approaches to quantitative structure-activity techniques. A further purpose was to bring together acknowledged and recognized workers in this area and to encourage them to focus on the problems of handling antitumor data. In order to provide some background to each of the speakers and discussion leaders, they were provided in advance with examples of the chemical and biological data available to the NCI.

There are four major approaches to quantitative structure-activity relationships (QSAR) now being employed by institutions around the country. These are: (1) the extrathermodynamic approach, exemplified by the Hansch technique, (2) the extrathermodynamic approach, utilizing *de novo* sutstituent constants (the Free-Wilson technique), (3) the quantum chemical approach, and (4) pattern recognition. Accordingly, major proponents of each of these approaches were invited to speak. Further, another expert in each of the four areas was invited to lead a discussion on the potential application of that approach to antitumor data.

The symposium was well-received by staff members, contractors, and invited guests. Many of those attending expressed the opinion that the presentations were extremely informative and that future symposia would be useful and should be held in specific areas. The use of a committee of consultants were discussed. The problems of handling *in vivo* screening data in a quantitative approach to structure-activity was considered at length in the discussions.

In summary, the symposium provided a good perspective on those approaches which hold the most promise for use in the modification or design of better antitumor agents. Those approaches are, in order of probable potential to antitumor drug synthesis: (1) the extrathermodynamic approach (both Hansch and Free-Wilson), (2) pattern recognition, and (3) the quantum chemical technique. It is the intention of DDB to further evaluate each approach in detail and to consider the implementation of various QSAR techniques in the design of new or improved antitumor agents.

Invited Participants:

Professor Corwin Hansch
Pomona College

Dr. John Topliss
Schering Corporation

Dr. William Purcell
The University of Tennessee

Dr. Stefan Unger
Pomona College

Dr. Joyce Kaufman
Johns Hopkins University

Dr. Eric Lien
University of California

Dr. Richard Cramer
Smith Kline & French

Dr. Yvonne Martin
Abbott Laboratories

Dr. Paul Craig
The Franklin Institute

Dr. Bruce Kowalski
The University of Washington

Dr. Ralph Christoffersen
The University of Kansas

Dr. Takeru Higuchi
The University of Kansas

Dr. Ralph Notari
Ohio State University

II. Quinones as Anticancer Agents

A one day symposium devoted to 'Quinones as Anticancer Agents' was held on December 7, 1973, at N.I.H. The quinone function is found in several clinically important antitumor drugs (e.g. adriamycin, mitomycin C). The objectives of this symposium were (1) a determination of the general importance of the quinone group to antitumor activity, (2) a discussion of the current research efforts in this area, and (3) a determination of those areas of quinone chemistry which might hold future promise. Six speakers addressed themselves to quinone structure-antitumor activity relationships, synthetic progress in the adriamycin area, clinical trials with lapachol, quinone natural products, the pharmacology of Coenzyme Q, and the synthesis and pharmacology of bioreductive antitumor agents. Several conclusions were reached. Among these were (1) natural products, especially fermentation products, have been the best source of useful quinone antitumor agents. Adriamycin currently shows the most promise among these materials; (2) aziridinyl compounds are the most active synthetic family in the quinone group; (3) several potentially useful new areas are being explored in the synthesis of adriamycin analogs, and (4) Coenzyme Q might have utility in combination with adriamycin to reduce the latter agents toxicity.

Over fifty people attended the symposium. A number of favorable comments were received, especially as related to the wide diversity of disciplines covered and, in general, the usefulness of an interdisciplinary approach to this type of investigation. In addition to N.I.H. attendees, invited participants included the following:

Dr. A. Sartorelli
Yale University

Dr. A. J. Lin
Yale University

Dr. K. V. Rao
University of Florida

Dr. K. Folkers
University of Texas

Dr. D. W. Henry
Stanford Research Institute

Dr. C. Hansch
Pomona College

Dr. J. Montgomery
Southern Research Institute

Dr. G. R. Pettit
Arizona State University

Dr. C. C. Cheng
Midwest Research Institute

Dr. E. Hodnett
Oklahoma State University

Dr. J. Blankley
Parke-Davis & Company

Dr. M. Foster
Starks Associates

III. Synthesis of Adriamycin and its Analogs

A Working Session on 'The Synthesis of Adriamycin and its Analogs' was held at N.I.H. on July 2, 1973. Approximately 30 people participated in this conference which had the objectives: (1) a discussion of the state-of-the-art in adriamycin synthesis; and (2) suggestions for future investigations. Eight speakers briefly described the clinical utility of the drug, progress towards the synthesis of the aglycone and sugar portions of the molecule, the aglycone-sugar coupling reaction, potential promising analogs which should be prepared, and other antibiotics structurally related to adriamycin.

It was generally agreed that an intense effort should be mounted in the synthesis of adriamycin analogs and derivatives as soon as possible. Several suggestions for new synthetic work was made. A practical synthesis for the aglycone was considered to have top priority.

In addition to N.I.H. investigators, invited participants included:

Dr. Emil Frei
Children's Cancer Research Foundation

Dr. Leon Goodman
University of Rhode Island

Dr. Corwin Hansch
Pomona College

Dr. David Henry
Stanford Research Institute

Dr. Derek Horton
Ohio State University

Dr. Andrew Kende
University of Rochester

Dr. Edward Modest
Children's Cancer Research Foundation

Dr. G. Robert Pettit
Arizona State University

Dr. Fred Starks
Starks Associates, Inc.

IV. Tetrahydrouridine

On August 2, 1973 a conference was held to review the current status of Tetrahydrouridine (THU) (NSC-112907). This conference was held at N.I.H. and was chaired by Dr. Seymour Cohen, University of Colorado School of Medicine. Presentations were made relating to the background and rationale for using THU, comparative screening with and without THU, biochemical studies, pharmacology, toxicology, clinical potential, chemical production, chemical purity and stability, and potential cost. Twenty-six individuals attended the conference and there were considerable differences in opinion expressed as to the need and advisability for further work with this material and also as to its potential. A majority of the invited participants questioned the advisability and need (at least until the evaluation of such agents as cyclocytidine has been completed) for further contract effort with this agent. The problems involved with the production, stability and purity of the agent were discussed. It was concluded that the material currently on hand should be purified and made available for possible further study.

Invited Participants

Dr. Seymour Cohen (Chairman)
University of Colorado
School of Medicine

Dr. Bruce Mellett
Southern Research Institute

Dr. Frank Maley
New York State Dept. of Health

Dr. Alan Sartorelli
Yale University School of Medicine

Dr. John Montgomery
Southern Research Institute

Dr. Milan Slavik
National Cancer Institute

Dr. John Venditti
National Cancer Institute

Dr. Gary Neil
The Upjohn Company

Dr. Jack Fox
Sloan-Kettering

Dr. Robert Jagow
Aerojet General Corporation

Dr. Peter Lim
Stanford Research Institute

Dr. David Cooney
National Cancer Institute

Dr. Robert Engle
National Cancer Institute

Dr. Harry B. Wood, Jr.
National Cancer Institute

V. Nucleosides

A review of the nucleoside area was held on June 21, 1973 at N.I.H. The purpose of the review was (1) whether or not synthetic effort should continue to be devoted to the preparation of nucleosides and (2) if so, what areas should be emphasized and/or de-emphasized. The major emphasis of the review was on the clinical utility of nucleosides since previous nucleoside reviews had stressed the chemical and biochemical aspects of nucleosides. Five formal presentations were made covering a clinical approach to analog development with emphasis on the nucleoside area, a critical review of clinical use of nucleosides, clinical action of nucleosides with emphasis on arabinosyl derivatives, biochemical interactions of nucleosides, and the design and synthesis of nucleoside analogs. The consensus of those present was that there is a future for nucleosides in cancer chemotherapy and that a synthetic program in this area should be maintained. Due note was taken that nucleosides have shown very little activity against solid tumors; however, it was also noted that there has been only limited evaluation to date of nucleosides against such tumors in man. It was felt that new and better nucleosides were needed and possible. The opinion was expressed that the areas of nucleosides provide the greatest potential for new, effective antimetabolites against cancer and that future advances in the chemotherapy of cancer at the clinical level rests in drug combinations of which one of the most promising types is nucleoside antimetabolites with other agents.

Invited Participants

Dr. Emil Frei
Children's Cancer Research Foundation

Dr. William Creasey
Yale University School of Medicine

Dr. Alan Sartorelli
Yale University School of Medicine

Dr. David Henry
Stanford Research Institute

Dr. Alex Bloch
Roswell Park Memorial Institute

Dr. Joseph Burchenal
Sloan-Kettering

Dr. Stephen Carter
National Cancer Institute

Dr. John Montgomery
Southern Research Institute

Dr. Lee Bennett
Southern Research Institute

Dr. Leroy Townsend
University of Utah

In addition to the above symposiums the following seminars were sponsored by the Drug Development Branch:

Recent Work on the Synthesis of Some Seven-Membered Ring Compounds by Dr. George R. Proctor, University of Strathclyde, Glasgow, Scotland

L-Phenylalanine Ammonia Lyase Therapy of Neoplasia by Dr. Creed W. Abell, The University of Texas Medical Branch, Galveston, Texas

Synthesis and Structure Determination of Some Ribofuranonucleosides by Dr. Jean-Louis Imbach, Biorganic Chemistry Laboratory, USTL, Montpellia, France

Some Organometalic Compounds with Biological Interest by Dr. G. J. M. van der Kerk, Institute for Organic Chemistry, TNO, Croesestraat, Utrecht, Netherlands

Chemistry of Pyrazole and Indole Analogs of Purine Nucleosides by Dr. Maria Preobrazhenskaya, Institute of Experimental and Clinical Oncology USSR Academy of Medical Sciences, Moscow, Russia

The Streptovaricins and Geldanamycin; Chemistry, Biological Activity and Biosynthesis by Professor Kenneth L. Rinehart, Jr., University of Illinois, Urbana, Illinois

Inhibition of Biosynthetic Processes and Loss of Transplantability of Cancer Cells with New Type of SH Combining Agents by Professor L. Drobnica, University of Pennsylvania, Philadelphia, Pennsylvania

Sulfhydryl Group Addition and Elimination in Enzymatic Catalysis: The Mechanism of Action of the Thymidylate System by Dr. Thomas I. Kalman, The State University of New York at Buffalo, Buffalo, New York

Worldwide Surveillance

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries, grantees and academic institutions, research organizations and other governmental laboratories, since a substantial number of compounds are contributed to the Program on a voluntary basis from these sources. Good public relations must be and have been maintained by reporting of data and by making special handling of unusual agents a matter of normal service. With the increasing complexity of data processing and growth of the Program, constant awareness of the problems of 'Commercial Discreet' data must ever be present. A total of 2804 individual suppliers, of which 1244 are industrial suppliers, have contributed samples to the Program. During 1973, there have been 162 new non-industrial suppliers and 100 new industrial suppliers submitting materials for primary screening. Even though the majority of these contributors are domestic suppliers many materials have been supplied from foreign countries. The increasing number of contributors is an index of the continued enthusiasm for the Program by the scientific community.

The Branch's continuous international surveillance for the acquiring new agents, synthetic and natural products, has provided over 344 foreign suppliers from industry, academic institutions and governmental and research organizations. Close contact is maintained with these and other potential new suppliers to provide interchange of chemical data and biological information on potential chemicals and drugs of interest to NCI and the scientific community-at-large. International visits were made by Dr. Harry B. Wood, Jr. during this period to the following:

Drs. Housley, Cliffe, Titman, Wolffe, Lewis, Cobb, Simpson, Boots Co.,Ltd.
Nottingham, England

Drs. Symington, Connors, Ross, Foster, Davis, Chester Beatty Research
Institute, London, England

Dr. Williams, St. Andrew University (met at Chester Beatty)

Dr. Lewis Smith, Hoffman LaRoche (met at Chester Beatty)

Dr. Bridgwater, Maybridge Chemical Company (met at Chester Beatty)

Drs. Dickenson, Simister, Ward Blenkinsop Pharmaceuticals, Ltd., London,
England

Dr. Heyes and Mr. Price, Beecham Research, Betchworth, Surrey, England

Drs. Kroneberg, Schlumberger, Bierling, Schutz, Truscheit and Brenner,
Bayer, Wuppertal- Elberfeld, Germany

Drs. Tagnon, Kenis, Mousin, Atassi, Hildebrand, Blank, Wineberg, University
of Groningen, Holland (met at Jules Bordet), Jules Bordet, Brusells

Professor Garattini, Mario Negri, Milan, Italy

Drs. Ghione, Rivalta, Arcamone and Scurati-Manzoni, Farmitalia, Montecatini Edison, Milan, Italy

Accomplishments

The Drug Development Branch provided a steady flow of materials into the Program. These materials consisted of chemicals, plant products, marine and insect products and fermentation products. Some of these materials have demonstrated confirmed activity in one or more of the primary screens (Step III) and have been assigned as candidates for preclinical toxicological evaluation (Decision Point II). A number of Investigational New Drug Applications (IND) have been submitted to the Food and Drug Administration (see Table I).

In comparing 1973 with 1972, the data in Table I reflects an increase in the number of crude plant extracts entering the Program. The number of crude extracts in the fermentation product category decreased slightly while there has been a slight increase in the number of animal products. There has been a major increase in the number of synthetic materials entering the Program.

Since the greatest number of active materials in the past has come from the synthetic category, it is expected that as the input in the Program increases, the total number of active materials will also increase. This has been the case as shown in Table I. There were 931 confirmed activities in 1973, an increase of 556 in comparison with 1972. Of these 931 confirmed actives, 907 were synthetic, 7 plant products and 17 fermentation products. During 1973 there was an increase of 1 agent over 1973 passing Decision Point II (approved for toxicology and as candidates for clinical trial). Of the 10 new Decision Point II compounds, 9 were synthetic, 1 was a plant product and there were no fermentation or animal products. There were 14 IND drugs filed for clinical use with the FDA. Of the 14 there were 11 synthetic, 1 was a plant product, and 2 were fermentation products. There is every reason to believe these numbers will increase significantly during 1974.

Of the 34,434 new crystalline materials added in 1973 to the Program, 1332 compounds (3.9%) were acquired under contract, 878 compounds (2.5%) were purchased, 19845 compounds (57.6%) were voluntarily submitted from industrial sources, 12379 compounds (36.0%) were from non-industrial suppliers. Of the total, 15390 new materials were acquired by field collection trips. Of the 34434 new crystalline materials added to the program during this report period, 34143 were synthetics. There were 907 confirmed active synthetics, 9 synthetics were selected for preclinical toxicology and IND's were filed on 11 synthetic materials (Table I).

Of the 11661 plant extracts tested, approximately 82% were from contractors, and 222 crude extracts were active. One hundred and fifty four new crystalline materials were isolated from plant extracts and 7 have shown confirmed activity (Step III). During this period one material of plant origin was selected for preclinical toxicological evaluation and one IND was filed (Table I).

From 2550 fermentation extracts, 267 demonstrated activity. One hundred and thirty-six were in crystalline form and 17 have shown confirmed activity (Step III). No fermentation products were selected for preclinical toxicological evaluation (Decision Point II), and two IND's were filed (Table I).

A relatively new area of exploration in the search for new drugs has been the investigation of animal products. Of the 2689 crude animal products, 83 have shown activity. One crystalline material has been isolated during this period, none has shown confirmed activity (Step III) and none were approved for Decision Point II or filed for an IND (Table I). There is every reason to believe that this new area will provide unique drugs for use in treating cancer.

The area of development and production of dosage forms for clinical use has continued to function at a high level of effort. In 1973, 25 projects for formulation development were assigned to the pharmaceutical contractors. Many of these projects have been completed and the resultant experimental dosage forms have been submitted for preclinical and clinical evaluation. Nine of these dosage forms were cleared for clinical distribution. Additionally, modifications requiring IND supplements were made on ten existing formulations.

The production record achieved by formulation contractors accounts for 419,655 compressed tablets; 835,350 hard gelatin capsules and 458,377 vials. A total of 1,339,344 drug items (vials and bottles) were received in 1973 and 1,131,801 items were distributed to clinical investigators throughout the world during the same period. An average inventory of 129 different compounds involving approximately 214 different formulations has been maintained.

In certain situations it is desirable to evaluate more than one drug or a drug and placebo under the same conditions. This is done by 'double-blind' clinical studies which require that the materials used are to be formulated in dosage forms that cannot be differentiated from each other except by code. There are thirteen coded studies on inventory.

The Branch upon request, has reviewed and commented upon 150 Grant Proposals. The opinions expressed have been used extensively by the Grant Study Sections in their evaluation of the Proposals. Such comments may include the number of materials submitted for testing by the grantee, indicating those compounds showing promise as potential antitumor agents.

Also upon request, the Branch has reviewed 5 patent applications. The applications are reviewed as to novelty, expected utility and need to protect the public's interest by patent. The opinions and recommendations are used by the NIH Patent Branch in their consideration of the application.

The Branch manages a total of 56 contracts with industrial organizations, academic and research institutions, both domestic and foreign, totaling approximately 11 million dollars and covering the major areas as shown under the primary responsibilities of the Branch. Of the 56 contracts, 53 are domestic and three are foreign contracts.

The total contract funding for all of Drug Development Branch related tasks during FY 74 was 11,037,282. Table IV provides a breakdown of these funds categorized by the Division of Cancer Treatment's Linear Array Code. The Branch projects showing the distribution of effort among the contractors are given in Table V. The contractor's effort among various Branch projects is given in Table VI.

Summary of Branch Achievements

In summary, the following were achieved during the calendar year of 1973 by the Drug Development Branch:

1. A total of 51334 new substances (Table I) was obtained for screening or other evaluation;
    a. 34143 synthetics
    b. 2550 antibiotic cultures
    c. 11661 plant materials
    d. 2689 animal materials
    e. 290 other crystalline materials
2. A total of 1527 compounds were prepared under contract;
    a. 195 were resynthesized by the preparation laboratory contracts
    b. 1281 were synthesized as new compounds from synthesis and prep lab contracts
    c. 51 were from the natural product laboratory contracts
3. Ten new substances were assigned to Decision Point II (Table I and II) (Candidates for preclinical toxicology);
    a. 1 plant product
    b. 9 synthetic compounds
4. Fourteen drugs were assigned IND numbers (Table I and III)
    a. 2 fermentation products

   b. 1 plant product
   c. 11 synthetic compounds

5. Nine hundred thirty-one compounds were assigned to Step III (Table I);

6. Analytical-Quality control determinations were made on 507 different lots of 185 different compounds represetning 355 lots of bulk compounds and 152 lots of clinical formulations;

7. Twenty radiolabeled compounds (Table IX) were procured;

8. Four new dosage forms were developed and five existing dosage forms were modified;

9. Approximately 4,857,795 formulated units were procured;
   a. 2,619,365 compressed tablets
   b. 964,650 hard gelatin capsules
   c. 1,273,780 vials

10. Fifty-six contracts (some joint contracts) were supervised by the Branch;
    a. 18 synthesis laboratories
    b. 8 preparation laboratories
    c. 9 plant and animal products
    d. 4 drug formulations
    e. 6 fermentation products
    f. 2 analytical-quality control
    g. 2 radiolabel laboratories
    h. 7 miscellaneous

11. There were 150 Grant Proposals and 5 Patent Applications reviewed and commented upon;

12. Samples were received from 100 new industrial concerns and 162 new non-industrial sources;

13. Sixty-six manuscripts by contractors were cleared for publication and seven manuscripts were published by the Branch staff;

14. Five scientific symposia and eight seminars were sponsored.

TABLE I

MATERIALS ACCESSIONED AND ACTIVITY REPORT

	CRUDE EXTRACTS NO. SUBMITTED		CRUDE EXTRACTS NO. ACTIVE		CRYSTALLINE MATERIAL SUBMITTED		STEP III(2)		DECISION POINT II(3)		IND FILED	
	1972	1973	1972	1973	1972	1973	1972	1973	1972	1973	1972	1973
Fermentation Products	2712	2550	204	267	28	136	3	17	--	--	1	2
Animal Products	1669	2689	54	83	--	1	--	--	--	--	--	--
Plant Products	10434	11661	189	222	108	154	16	7	4	1	1	1
Synthetics	--	--	--	--	15634	34143	356	907	5(5)	9(6)	3(6)	11
TOTAL	14815	16900	447	572	15770	34434	375	931	9	10	5	14

(1) Based on calendar year 1973.

(2) Showing confirmed activity.

(3) Selected as candidates for preclinical toxicological evaluation and clinical trial.

(4) Individual species.

(5) One was a synthetic modification of a fermentation product.

(6) One was a synthetic modification of plant material.

TABLE II

COMPOUNDS PASSING DECISION NETWORK II A

NSC NO.	COMPOUND NAME	SOURCE
51143	1*H*-Imidazo[1,2-*b*]pyrazole, 2,3-dihydro-	Synthetic
73754	Uracil,5-[(2-chloroethyl)-(2-fluoroethyl)amino]-6-methyl- (Fluorodopan)	Synthetic
D-124921		Synthetic
136020E	Androst-4-en-3-one, 17β-hydroxy-7β-methyl-, propionate (Testosterone, 7β-methyl-, propionate)	Synthetic
151466E	Triethylamine, 2-[*p*-(2-chloro-1,2-diphenylvinyl)phenoxy]-, citrate(1:1), (*Z*)- (Clomifene, *cis*-, citrate)	Synthetic
154020	1,4,5,6,8-Pentazaacenaphthylene, 3-amino-1,5-dihydro-5-methyl-1-β-*D*-ribofuranosyl-	Synthetic
154890	Berbinium, 5,6,7,8,13,13a-hexadehydro-2,3,10,11-tetramethoxy-8-methyl-, salt with acetic acid monoanhydride with sulfuric acid (Coralyne, sulfoacetate)	Synthetic
165563	Bruceantin	Plant
167780	Leucine, *N*-[*N*-acetyl-3-[*p*-[bis-(2-chloroethyl)amino]phenyl]-DL-alanyl]-, ethyl ester, *L*- (Asaley)	Synthetic
167781	Benzamide, *N*-[bis(1-aziridinyl)-phosphinyl]-2,5-diiodo- (Diiodobenzotepa)	Synthetic

TABLE III

IND'S FILED

NSC NO.	COMPOUND NAME	SOURCE
15200	Gallium nitrate	Synthetic
71851	9*H*-Purine-6-thiol, 2-amino-9-(2-deoxy-α-*D*-*erythro*-pentofuranosyl)-	Synthetic
77037	Tetrandrine, (+)-	Plant
83265	*L*-Alanine, 3-(tritylthio)-	Synthetic
104801	Acrylic acid, 3-*p*-anisoyl-3-bromo-, sodium salt (Cytembena)	Synthetic
118994	Digylcolaldehyde, α-(hydroxymethyl)-α'-(6-hydroxy-9*H*-purin-9-yl)-	Synthetic
135758	2,5-Piperazinedione, 3,6-bis(5-chloro-2-piperidyl)-, dihydrochloride	Synthetic
139105	Ethanesulfonic acid, compd. with α-[2-chloro-4-(4,6-diamino-2,2-dimethyl-*s*-triazin-1(2*H*-yl)phenoxy]-*N*,*N*-dimethyl-*m*-toluamide (1:1)	Synthetic
143769	*Mycobacterium* *tuberculosis*, methanol extraction residue of (MER of BCG)	Fermentation
145668	6*H*-Furo[2',3':4,5]oxazolo-[3,2-*a*]pyrimidine-2-methanol, 2,3,3a,9a-tetrahydro-3-hydroxy-6-imino-, monohydrochloride $O^{2,2'}$-Cyclocytidine, monohydrochloride	Synthetic
148958	Uracil, 5-fluoro-1-(tetrahydro-2-furyl)- (Ftorafur)	Synthetic
151466E	Triethylamine, 2-[*p*-(2-chloro-1,2-diphenylvinyl)phenoxy]-,citrate (1:1), (*Z*)- (Clomifene, *cis*-, citrate)	Synthetic

TABLE III (Continued)

NSC NO.	COMPOUND NAME	SOURCE
167780	Leucine, N-[N-acetyl-3-[-bis(2-chloroethyl)amino]phenyl]-OL-alanyl]-,ethyl ester,L- (Asaley)	Synthetic
B116327	Mycobacterium tuberculosis (BCG)	Fermentation

TABLE IV

DISTRIBUTION OF DDB CONTRACT PROJECTS BY DCT-ARRAY[1]

ARRAY	LINEAR L	SUPPLEMENTARY S	EXPLORATORY E	TOTAL	PERCENT
IXX	$6,969,032	0	$39,198	$7,008,230	63.50
2B1	176,744	0	0	176,744	1.60
2B4	1,557,459	0	0	1,557,459	14.11
3X2	2,294,849	0	0	2,294,849	20.79

PROJECTS

ARRAY CODE	PROJECT	FUNDING	PERCENT
L-IXX	Synthetics	$2,789,273	25.27
	Plant Products	944,921	8.56
	Fermentation	1,596,446	14.47
	Marine & Anthropods	48,600	0.44
	Storage & Distribution	172,200	1.56
	Data Processing	1,417,592	12.85
E-1XX	Structure-Activity	39,198	0.36
L-2B1	Formulation-Research & Development	176,744	1.60
L-2B4	Procurement	1,064,366	9.64
	Quality Control	103,598	0.94
	Radiolabel Procurement	327,995	2.97
	Storage & Distribution	61,500	0.56
L-3X2	Production	1,191,921	10.80
	Formulation Production	594,238	5.38
	Quality Control	414,392	3.75
	Radiolabel Procurement	81,998	0.74
	Storage & Distribution	12,300	0.11
	TOTAL	$11,037,282	100.00%

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[1] Funding based on amount negotiated for FY '74, if any, or on amount obligated in FY '73 as of March 31, 1974 DCT Monthly Report of Contract Commitments, or on new or renewal proposed budget.

TABLE V

DDB PROJECTS - DISTRIBUTION OF EFFORT AMONG CONTRACTORS

PROJECT (LA Code)	CONTRACTOR (CONTRACTOR NO.)				FUNDING
I. New Agent Acquisition					TOTAL: $5,418,438
A. Synthetics:				$2,789,273	
Collections: (L-1XX)					
	Chester Beatty Research Inst.	(43736)	$ 20,000		
	Mario Negri	(33720)	29,898		
	Starks C.P., Inc.	(23705)	167,603		
Synthesis: (L-1XX)					
	Ash Stevens, Inc.	(12314)	72,098		
	U. British Columbia	(23223)	57,678		
	Central Drug Res. Inst. (India)	(PL-480)	8,098		
	Collaborative Research, Inc.	(60506)	89,557		
	Collaborative Research, Inc.	(12326)	51,000		
	Dow Chemical Company	(12313)	90,509		
	Dow Chemical Company	(33724)	142,198		
	University of Georgia	(12311)	36,574		
	Massachusetts Institute of Tech.	(43712)	55,058		
	University of Michigan	(92004)	50,000		
	Microbiological Assoc., Inc.	(33728)	312,000		
	Midwest Research Institute	(33743)	324,220		
	Pennwalt Corporation	(12309)	60,000		
	Univ. of Rhode Island	(12312)	39,450		
	Sheehan Institute for Res.	(23719)	53,016		
	Southern Research Institute	(23712)	561,000		
	Stanford Research Institute	(33742)	413,338		
	Upjohn Company	(23709)	35,978		
	University of Utah	(23710)	120,000		

B. Plant Products:				$ 944,921
Collections:	(L-1XX)			
U. S. Dept. of Agriculture		(40001)	$286,180	
Central Drug Res. Inst. (India)		(PL-480)	16,196	
University of Hawaii		(33747)	65,000	
Extraction & Isolation:	(L-1XX)			
University of Arizona		(33750)	101,905	
Central Drug Res. Inst. (India)		(PL-480)	24,294	
University of Illinois		(22078)	90,000	
Research Triangle Institute		(92019)	130,150	
University of Virginia		(12099)	150,000	
WARF Institute, Inc.		(43707)	81,196	
C. Fermentation:	(L-1XX)			$1,596,446
Bristol-Myers Company		(90035)	283,560	
Bristol-Myers Company		(43759)	163,220	
Georgetown University		(43732)	43,954	
University of Illinois		(23208)	68,252	
Parke-Davis & Company		(43754)	448,060	
Upjohn Company		(43753)	589,400	
D. Marine and Anthropods:				$ 48,600
Collections:	(L-1XX)			
Arizona State University		(12308)	15,552	
Extraction & Isolation	(L-1XX)			
Arizona State University		(12308)	33,048	
E. Miscellaneous:	(E-1XX)			$ 39,198
Pomona College		(33702)	39,198	

II. Procurement of Material

TOTAL: $2,666,280

A. Procurement for Pre-Clinical: (L-2B4) $1,064,366

Aerojet-General Corporation	(81300)	$ 38,000
Aldrich Chemical Company, Inc.	(23706)	122,500
Dow Chemical Company	(12165)	59,998
Merck & Company	(22002)	387,516
Monsanto Research Corporation	(33709)	26,724
Parke-Davis & Company	(23708)	133,491
Pharm-Eco	(23707)	71,800
Starks Associates, Inc.	(23203)	203,437
University of Virginia	(12099)	20,900

B. Production: Toxicological & Clinical Studies (L-3X2) $1,191,921

Aerojet-General Corporation	(81300)	152,000
U. S. Dept. of Agriculture	(40001)	62,820
Aldrich Chemical Company, Inc.	(23706)	52,500
Dow Chemical Company	(12165)	140,000
Merck & Company	(22002)	129,172
Monsanto Research Corporation	(33709)	240,513
Parke-Davis & Company	(23708)	133,491
Pharm-Eco	(23707)	107,700
Starks Associates, Inc.	(23203)	135,625
University of Virginia	(12099)	38,100

C. Radiolabel - Pre-Clinical (L-2B4) $ 327,995

Monsanto Research Corporation	(23715)	168,000
Stanford Research Institute	(12162)	159,995

D. Radiolabel - Clinical (L-3X2) 81,998

Monsanto Research Corporation	(23715)	42,000
Stanford Research Institute	(12162)	39,998

III.	Analytical-Quality Control				TOTAL: $ 517,990
A.	Procurement for Pre-Clinical:	(L-2B4)		$ 103,598	
	Midwest Research Institute	(33722)	$ 40,000		
	Stanford Research Institute	(33723)	63,598		
B.	Production for Clinical Studies:	(L-3X2)		$ 414,392	
	Midwest Research Institute	(33722)	160,000		
	Stanford Research Institute	(33723)	254,392		
IV.	Drug Formulation				TOTAL: $ 770,982
A.	Research & Development:	(L-2B1)		$ 176,744	
	Ben Venue Laboratories, Inc.	(43701)	54,002		
	University of Kansas	(23217)	90,000		
	Philips Roxane Laboratories	(12139)	13,992		
	University of Tennessee	(02084)	18,750		
B.	Production:	(L-3X2)		$ 594,238	
	Ben Venue Laboratories, Inc.	(43701)	486,021		
	Philips Roxane Laboratories	(12139)	51,967		
	University of Tennessee	(02084)	56,250		
V.	Storage & Distribution				TOTAL: $ 246,000
A.	New Agents:	(L-1XX)		$ 172,200	
	Microbiological Associates, Inc.	(33728)	172,200		
B.	Pre-Clinical:	(L-2B4)		$ 61,500	
	Microbiological Associates, Inc.	(33728)	61,500		
C.	Toxicological & Clinical:	(L-3X2)		$ 12,300	
	Microbiological Associates, Inc.	(33728)	12,300		

VI. Data Processing: (L-1XX) TOTAL: $1,417,592

			$1,417,592
Chemical Abstract Service	(51021)	$350,952	
Chemical Abstract Service	(43722)	482,500	
Starks C.P., Inc.	(23705)	71,830	
University of Pennsylvania	(33719)	512,310	

Table VI

DDB CONTRACTORS, Distribution of Effort Among Projects

CONTRACTOR (Contract Number) Projects Monitored by Drug Development Branch	Funding of Tasks Monitored by Drug Development Branch Distribution by Project (Code)	(%)	($)
Aerojet-General Corp. (81300)		100	190,000
Procur. for pre-clin. studies	L-2B4	20	38,000
Prod. for tox. and clin. studies	L-3X2	80	152,000
USDA (40001)		100	349,000
Acq. new agents-plant materials	L-1XX	82	286,180
Prod. for tox. and clin.-plant materials	L-3X2	18	62,820
Aldrich Chemical Co. (23706)		100	175,000
Proc. for pre-clinical studies	L-2B4	70	122,500
Prod. for tox. and clin. studies	L-3X2	30	52,500
Arizona U. of (33750)*		89	101,905 114,500
Acq. new agents-plant ext. & isol.	L-1XX	89	101,905
Arizona State U. (12308)		100	48,600
Acq. new agents-marine & anthropod coll.	L-1XX	32	15,552
Acq. new agents-marine & antrhopod ext. & isol.	L-1XX	68	33,048
Ash Stevens, Inc. (12314)		100	72,098
Acq. new agents-synthesis	L-1XX	100	72,098
Ben Venue Labs., Inc. (43701)		100	540,023
Experimental form. devel.	L-2B1	10	54,002
Form. prod. for clin. studies	L-3X2	90	486,021
Bristol-Myers Co. (90035)*		62	283,560 457,354
Acq. new agents-fermentation	L-1XX	62	283,560
Bristol Myers Co. (43759)		100	163,220
Acq. new agents-fermentation	L-1XX	100	163,220
British Columbia, U. of (23223)		80	57,678 83,984
Acq. new agents-synthesis	L-1XX	80	57,678

Table VI

CDRI (India) (PL480)*		90	48,588
			53,988
Acq. new agents-synthesis	L-1XX	15	8,098
Acq. new agents-plant coll.	L-1XX	30	16,196
Acq. new agents-plant ext. & isol.	L-1XX	45	24,294
Chemical Abstracts Service (51021)		100	350,952
Acq. new agents-data processing	L-1XX	100	350,952
Chemical Abstracts Service (43722)		100	482,500
Acq. new agents-data processing	L-1XX	100	482,500
Chester Beatty Research Institute (43736)		100	20,000
Acq. new agents-syn. coll.	L-1XX	100	20,000
Collaborative Research, Inc. (60506)		100	89,557
Acq. new agents-synthesis	L-1XX	100	89,557
Collaborative Research, Inc. (12326)		100	51,000
Acq. new agents-synthesis	L-1XX	100	51,000
Dow Chemical Co. (12313)		100	90,509
Acq. new agents-synthesis	L-1XX	100	90,509
Dow Chemical Co. (33724)		100	142,198
Acq. new agents-synthesis	L-1XX	100	142,198
Dow Chemical Co. (12165)		100	199,998
Procur. for pre-clin. studies	L-2B4	30	59,998
Prod. for tox. & clin. studies	L-3X2	70	140,000
Georgetown University (43732)		100	43,954
Acq. new agents-fermentation	L-1XX	100	43,954
Georgia U. of (12311)		100	36,574
Acq. new agents-synthesis	L-1XX	100	36,574
Hawaii, U. of (33747)		100	65,000
Acq. new agents-plant coll.	L-1XX	100	65,000
Illinois, U. of (22078)		100	90,000
Acq. new agents-plant ext. & isol.	L-1XX	100	90,000
Illinois, U. of (23208)		100	68,252
Acq. new agents-fermentation	L-1XX	100	68,252

Table VI

Kansas, U. of (23217)		100	90,000
Experimental form. devel.	L-2B1	100	90,000
Mario Negri (33720)*		20	29,898
			149,490
Acq. new agents-syn. coll.	L-1XX	20	29,898
Massachusetts Institute of Technology (43712)		100	55,058
Acq. new agents-synthesis	L-1XX	100	55,058
Merck & Co. (22002)		100	516,688
Procur. for pre-clin. studies	L-2B4	75	387,516
Prod. for tox. and clin. studies	L-3X2	25	129,172
Michigan, U. of (92004)		100	50,000
Acq. new agents-synthesis	L-1XX	100	50,000
Microbiological Assoc. Inc. (33728)*		37.4	558,000
			1,492,048
Acq. new agents-synthesis	L-1XX	20.9	312,000
Acq. new agents-storage & distrib.	L-1XX	11.6	172,200
Procur. for pre-clin.studies-storate & distrib.	L-2B4	4.1	61,500
Prod. for clin. studies-storage distrib.	L-3X2	0.8	12,300
Midwest Res. Inst. (33743)		94	324,220
			344,915
Acq. new agents-synthesis	L-1XX	94	324,220
Midwest Res. Inst. (33722)		100	200,000
Procur. for pre-clin. studies-qual.cont.	L-2B4	20	40,000
Prod. for clin. studies-qual.cont.	L-3X2	80	160,000
Monsanto Res. Corp. (23715)		100	210,000
Proc. for pre-clin. studies-radiolabel	L-2B4	80	168,000
Prod. for clin. studies-radiolabel	L-3X2	20	42,000
Monsanto Res. Corp. (33709)		100	267,237
Proc. for pre-clin. studies	L-2B4	10	26,724
Prod. for tox. & clin. studies	L-3X2	90	240,513
Parke, Davis & Co. (23708)		100	266,982
Proc. for pre-clin. studies	L-2B4	50	133,491
Prod. for tox. & clin. studies	L-3X2	50	133,491

Table VI

Parke, Davis & Co. (43754)		85	448,060 527,130
Acq. new agents-fermentation	L-1XX	85	448,060
Pennwalt Corp (12309)		100	60,000
Acq. new agents-synthesis	L-1XX	100	60,000
Pharm-Eco (23707)		100	179,500
Procur. for pre-clin. studies	L-2B4	40	71,800
Prod. for tox. & clin. studies	L-3X2	60	107,700
Philips Roxane Labs. (12139)		100	64,959
Experimental form. devel.	L-2B1	20	13,992
Form. prod. for clin. studies	L-3X2	80	51,967
Pomona College (33703)		100	39,198
Acq. new agents-strucutre-activity	E-1XX	100	39,198
Research Triangle Inst. (92019)*		95	130,150 137,000
Acq. new agents-plant ext. & isol.	L-1XX	95	130,150
Rhode Island, U. of (12312)		100	39,450
Acq. new agents-synthesis	L-1XX	100	39,450
Sheehan Inst. for Res. (23719)		100	53,016
Acq. new agents-synthesis	L-1XX	100	53,016
Southern Research Inst. (33712)		100	561,000
Acq. new agents-synthesis	L-1XX	100	561,000
Stanford Research Inst. (33723)		100	317,790
Procur. for pre-clin. studies-qual. cont.	L-2B4	20	63,598
Prod. for clin. studies-qual.cont.	L-3X2	80	254,392
Stanford Research Inst. (33742)		96	413,338 430,561
Acq. new agents-synthesis	L-1XX	96	413,338
Stanford Research Inst. (12162)		100	199,993
Procur. for pre-clin. studies-radiolabel	L-2B4	80	159,995
Prod. for clin. studies-radiolabel	L-3X2	20	39,998

Table VI

Starks Assoc. Inc. (23203)		100	339,062
Procur. for pre-clin. studies	L-2B4	60	203,437
Prod. for tox. & clin. studies	L-3X2	40	135,625
Starks C. P., Inc. (23705)		100	239,433
Acq. new agents-syn. coll.	L-1XX	70	167,603
Acq. new agents-data processing	L-1XX	30	71,830
Tennessee, U. of (02084)		100	75,000
Experimental form. devel.	L-2B1	25	18,750
Form. prod. for clin. studies	L-3X2	75	56,250
Upjohn Co. (23709)		100	35,978
Acq. new agents-synthesis	L-1XX	100	35,978
Upjohn Co. (43753)*		57	589,400 1,034,033
Acq. new agents-fermentation	L-1XX	57	589,400
Utah, U. of (23710)		100	120,000
Acq. new agents-synthesis	L-1XX	100	120,000
Virginia, U. of (12099)		100	209,000
Acq. new agents-plant ext. & isol.	L-1XX	72	150,000
Procur. for pre-clin. studies	L-2B4	10	20,900
Prod. for clinical trials	L-3X2	18	38,100
WARF (43707)		100	81,196
Acq. new agents-plant ext.	L-1XX	100	81,196

CHEMICAL AND DRUG PROCUREMENT SECTION

DRUG DEVELOPMENT BRANCH

Scope

The function of the Chemical and Drug Procurement Section is to synthesize new compounds for antitumor evaluation; to procure large quantities for advanced screening, confirmational testing, toxicologic and pharmacologic evaluation, and clinical trial in humans; to procure radiolabeled materials needed for pharmacologic and clinical studies; and to evaluate the materials being used in toxicologic, pharmacologic and clinical studies as to their identity, assay and purity.

The work of the Section is divided into the following three principal areas:

1. The Synthesis Activities, which provide new compounds for primary screening;

2. The Preparative Activities, which synthesize and isolates (a) bulk chemicals and drugs and (b) radiolabeled materials for toxicologic, pharmacologic and clinical investigations, for confirmational testing and for completion of primary evaluation; and

3. The Analytical-Quality Control Activities, which provide the analytical check on the identity, assay and purity of compounds and formulated drugs entering the toxicologic, pharmacologic and clinical programs. Solubility and stability data are also acquired as is structural analysis.

Staff

The staff of the Chemical and Drug Procurement Section consists of four professional staff and a secretary. One professional person is involved full time with the quality control activities of the Section. The professional staff members serve as project officers on the various contract activities of the Section as well as on other contracts that are monitored by the Drug Development Branch.

Synthesis Activities

The objective of the synthesis laboratories is to provide the program with new chemical agents for anticancer evaluations. The laboratories are designed to supply compounds of diverse structures and complexity. The compounds selected for preparation are based upon the following criteria:

1. Modification of clinically active compounds;

2. Leads generating from the screening program;

3. Biological and biochemical rationale;

4. Leads from the literature;

5. Compounds to explore structure-activity relationships;

6. New structural types; and

7. Agents to resolve questions on chemical and biochemical mechanism of action.

Even though major advances have been made in recent years, the treatment of cancer is still limited by the availability of useful agents. The usefulness of the majority of the present agents are limited by severe toxic side effects and/or are associated with the development of drug resistant tumors. In addition, many tumors do not respond to any of the current drugs. Therefore, a greater variety of agents with diverse structures and mechanism of action, broader tumor spectrum, and greater safety would be extremely valuable in cancer treatment. The problem of finding these improved drugs is the main objective of the contract synthesis program and its efforts are channeled into many areas of investigation. The following are some examples:

1. A significant amount of the synthetic effort of the Section has been directed toward the synthesis of natural products, their analogs and derivatives. Several products are not available in the quantities necessary for complete evaluation from natural sources, and if the structure is known, attempts are made to prepare the material by synthetic means where feasible. Many times the investigation of a synthetic route to the preparation of a natural product affords, or affords the means to, the preparation of analogs which may be more active and interesting than the natural material. It may also be desirable to prepare synthetic analogs and derivatives of naturally occurring material to develop the parameters of an interesting lead or in the hopes of reducing adverse toxicity or metabolism while retaining activity. Recognizing that natural materials are one source of interesting structures and compounds, quite often analogs and derivatives of these can be procured only by synthetic means.

   The clinically effective antibiotics daunorubicin and adriamycin are obtained in rather poor yields from the natural source. In addition, these compounds have dose limiting toxicologic (cardiac) properties and, therefore, synthetic work on the preparation of analogs and derivatives has continued at Stanford Research Institute. This work has been directed in the following main areas: (1) The preparation of a series of amino and carbonyl derivatives of the parent antibiotic since recent work has indicated that such derivatives may display reduced toxicity while retaining antitumor properties; (2) The preparation of more accessible synthetic analogs, mainly substituted naphthacenequinones and anthraquinones, with basic side chains that stimulate the aminosugar portion of the parent molecule; (3) The development of methodology in forming the glycosidic linkage and removal of the blocking groups on the sugar moiety; (4) The preparation of some synthetic aglycones; and (5) The total synthesis of adriamycin. During this report period the methodology of forming the glycosidic linkage and the deblocking of the resulting glucoside has been achieved. This appears to be a general procedure and work is in progress to couple the natural aglycones with other aminosugars, and to couple synthetic aglycones with the natural aminosugar. The work in this area is continuing and it is anticipated that the effort in this area will be increased during the coming year.

Methods for the synthesis of the clinically effective antibiotic streptozotocin have recently been developed and the large quantities of this agent needed by the program are now prepared via the synthetic route. These methods also make the modification of this interesting structure feasible. The major emphasis in this area has been on preparing analogs with modifications in the sugar portion of the molecule. Relatively few analogs of this interesting compound have been prepared and most of these have involved modifications in other than the sugar portion of the molecule. It has been speculated that the tissue specificity associated with streptozotocin is due largely to the nature of the sugar portion and by changing the sugar moiety one would expect to find significant differences in tissue specificity and/or antitumor activity. This work has been done at Southern Research Institute and the Upjohn Company.

A limited amount of synthetic effort is devoted to the preparation of some structural moieties of the antitumor antibiotic, Bleomycin. Bleomycin is a mixture of closely related antibiotics, the chemical structures of which have recently been elucidated. The bleomycins contain polypeptides composed of a substituted bithiazole among other moieties. A few bithiazoles and related thiazoles have been prepared for evaluation at Midwest Research Institute. The synthetic effort devoted to Bleomycin is being increased, including an approach to the total synthesis of the antibiotic. This work on the partial and total synthesis is being undertaken at Massachusetts Institute of Technology.

The antibiotic streptonigrin has demonstrated a high degree of antiviral activity in addition to antitumor activity and is obtained in rather poor yields from the natural source. Some synthetic effort is being devoted to developing a total synthesis of this material. In addition, some simplified synthetic analogs of streptonigrin have been and are being prepared at Midwest Research Institute which contain the major features of the parent molecule.

Some synthetic effort has been and is being devoted to the development of a general and versatile synthesis of the highly complex but clinically effective dimeric Vinca alkaloids, vincristine and vinblastine. In addition to the synthesis of these clinically effective alkaloids, the synthesis of related analogs and derivatives is also being undertaken. These analogs and derivatives should provide information concerning the mechanism of action of the Vinca alkaloids, their metabolism and structure-activity relationships. Also, a synthesis of these materials would provide the means of obtaining radiolabeled vincristine, vinblastine and related dimeric alkaloids for metabolism and drug distribution studies. The Vinca alkaloids, from the plant *Vinca rosea L*, are obtained via a very complex and costly extraction procedure. The compounds are present in only minute quantities in the plant. The development of a synthesis for these materials has the potential of not only providing the means of obtaining analogs, but of making the materials available in larger quantities and at a lower cost. This work is being done at the University of

British Columbia and a few analogs have been prepared and submitted for evaluation.

The recently completed total synthesis of the antitumor active alkaloids coralyne, nitidine and thalicarpine has made it possible to undertake the preparation of analogs of these three compounds which is being done at Midwest Research Institute. The analogs being prepared include the methylenedioxy derivative of coralyne, various methoxy derivatives of nitidine and the nitrogen isotere (nitrogen substituted for the oxygen in the ether linkage) of thalicarpine. A variety of salts of nitidine have been prepared in an attempt to improve the solubility property of the alkaloid. Other analogs of these three alkaloids are under investigation.

Several structural modifications of the L-1210 active alkaloid ellipticine have been prepared previously and some have demonstrated antitumor activity. The 9-hydroxy ellipticine is also in clinical trial in France. Additional derivatives have been prepared including a variety of N-6 substituents on three pyrido[4,3-b]carbazole nuclei. The N-substituents were chosen to provide a moderate degree of reactivity towards nucleophilic groups and to give a wide range of hydrophobic and hydrophilic character. Work in this area was done at Stanford Research Institute and is continuing.

In addition, some effort is being extended to the preparation of some analogs and derivatives of the interesting pyrrolizidine alkaloid, indicine-N-oxide and the biochemically interesting antibiotic, sparsomycin. Indicine-N-oxide is the only one of about 40 natural occurring such alkaloids which has shown activity in the L-1210 and P-388 leukemia test systems. The synthetic analogs are being prepared in an attempt to obtain other active derivatives and to determine the structural parameters for activity. This work is being done at Microbiological Associates, Inc.

A small amount of synthetic effort is being directed by the Upjohn Company towards the development of a synthesis of the antibiotic, AT-125, which is highly active against the L-1210 tumor system. AT-125 is produced by fermentation in very low yields.

2. The area of nucleosides has already provided a number of very interesting compounds capable of suppressing tumor growth in experimental animals and producing favorable clinical response in certain types of human cancer. Cytosine arabinoside and 5-FUDR are nucleosides which have demonstrated clinical activity. However, further chemical studies are needed to provide nucleosides of greater specificity and less toxicity and which are resistant to undesirable enzymatic degradation in the cancer patient.

   In addition, the study of nucleoside metabolism may be expected to contribute to the design of chemotherapeutic agents with antiviral activity against tumor-inducing viruses and may help elucidate many cellular processes in the synthesis of DNA and therefore in cell division. Nucleoside antimetabolites may function by inhibiting the incorporation of normal constituents in RNA or DNA and preventing the formation of enzymes necessary for biosynthesis of the tumor nucleic acids. Also, the conversion of nucleosides to nucleotides may be structurally less restrictive than the conversion of the free base to the corresponding nucleotides.

A wide variety of structural modifications and derivatives of nucleosides has been prepared by Southern Research Institute, Stanford Research Institute and the University of Utah and submitted for evaluation during this report period. These include (a) analogs of the nucleoside antibiotics such as tubercidin, toyocamycin, sangivamycin, formycin, formycin B, and puromycin, (b) tricyclic nucleosides, (c) nucleosides of pyrazolo-[3,4-d]pyrimidine, pyrazolo[4,3-d]pyrimidine, imidazo[4,5-c]pyridine and imidazo[4,5-b]pyrazine, (d) imidazole and benzimidazole nucleosides, (e) adenosylmethionine analogs, (f) "Carbocyclic sugar" nucleoside derivatives, (g) "carbon-carbon nucleosides", (h) deazapurine nucleosides and (i) nucleosides with natural bases and fraudulent sugars, with natural sugars and fraudulent bases, and with fraudulent bases and sugars. The synthetic effort devoted to the preparation of nucleosides has decreased during this report period; however, work in this area is continuing.

Some of the synthetic effort has been devoted to the preparation of analogs and derivatives of folic acid. Methotrexate is an important clinically effective antifolate. However, resistance develops to this agent sometimes rather rapidly which limits its effectiveness. Synthetic effort in this area has been directed towards developing an antifolate which is active against methotrexate resistant tumors and/or towards obtaining compounds which retain the activity and for which the resistance problem is eliminated. The structure of the folic acid molecule has been divided into five parts, (a) Ring A (pyrimidine) of the pteridine ring, (b) Ring B of the pteridine ring, (c) the Bridge, (d) the Benzene portion (Ring C), and (e) the Amino Acid moiety and modifications have been undertaken in each area. This work is being done at Southern Research Institute. The synthetic effort devoted to this area during this report period has been decreased considerably. New methodology needs to be developed for the preparation of this type of compound and steps have been taken to initiate work along these lines during the next year.

In reviewing the compounds which have shown activity in the program the largest majority of the compounds have contained nitrogen and many have been nitrogen heterocycles. Some of the synthetic effort is being devoted to the synthesis of new nitrogen heterocycles as potential antitumor agents. The emphasis in this area has been on the preparation of bridgehead nitrogen heterocycles, especially pyridazines, isoxazolo[4,5-b] pyrazines, pyrazolotriazines, pyrazolopyrimidines, pyrazolopyrazines, pyrazoloimidazopyrazines, imidazopyrazines, pyrrolopurines and indenopyrimidines. Several compounds of this type have been prepared and submitted for evaluation by Dow Chemical Company, the University of Rhode Island and the University of Michigan.

The class of compounds known as N-nitrosoureas are active against leukemia and some possess the ability to cross the blood-brain barrier. Three compounds of this class (BCNU, CCNU and Methyl CCNU), are currently undergoing clinical evaluation. Of special interest are the results from intracerebrally-inoculated L-1210 leukemia, Lewis Lung carcinoma, and the use of maximal single dose (one day only) treatment schedules. The testing in rodents with some compounds of this type has resulted in a significant number of cures based on thirty-day survivals. A few additional examples of this structural type have been synthesized by Southern Research Institute and submitted for antitumor evaluation.

6. A limited amount of effort has been devoted to the isolation and synthesis of metabolites of cyclophosphamide. This clinically effective agent is itself inactive and becomes effective only after appropriate activation in vivo. Work in this area is continuing at Collaborative Research, Inc., and Southern Research Institute in an effort to determine the mechanism of activation of this active agent and to isolate and synthesize the active metabolite(s).

7. Some of the synthetic effort of the Section has been directed towards the synthesis of compounds which potentially may localize in specific organs or parts of the body. The main effort in this area during this report period has been to develop CNS active antitumor agents. Some derivatives of ergocornine, a CNS active natural product in the lysergic acid family, have been prepared. In addition, some heterocyclic azepines and some acetylenic oxazolones and their ethynyl carbamate precursors have been prepared. The work in this area was done at the Sheehan Institute for Research and at the University of Georgia.

8. A limited amount of the synthetic effort has been directed towards the synthesis of potential antimetabolites. One such structural type is the triazene. Two imidazole-triazenes have demonstrated activity against L-1210 leukemia and are undergoing clinical evaluation. In addition, some compounds of this type have been shown to have broad spectrum antifungal and antibacterial activity. Thus, additional triazene derivatives have been prepared and are being synthesized by Southern Research Institute for evaluation as antitumor agents.

9. In addition to the effort devoted to the synthesis of nitrogen heterocycles as new structural moieties as potential antitumor agents, a limited amount of synthetic effort was devoted to the preparation of new sulfur/oxygen heterocycles. The emphasis in this endeavor has been to prepare some 1,2,4-trithiolanes, cyclic disulfides, trisulfides and related compounds. Several compounds of these types were prepared by Ash Stevens, Inc.

10. In order to develop the discovery of the high degree of antitumor activity in the coordination compound cis-diamminedichloroplatinum which is currently being studied clinically, the preparation of some coordination compounds of some of the transition metals has been initiated. Coordination compounds of platinum, rhodium, niobium, molybdenum, cobalt, iron and nickel have been prepared and are being evaluated in the standard tumor test systems. The Pennwalt Corporation is investigating this area.

11. The design and synthesis of oligo and polynucleotides as potential inhibitors of the RNA dependent DNA polymerases and other polymerases are under investigation. Some modified and unmodified deoxyoligonucleotide primers were prepared. These primers when complexed with the appropriate

polyribonucleotide templates to form primer-template duplexes may then act as inhibitors of the polymerases. This work was done at Collaborative Research, Inc.

Some synthetic effort has been devoted to the synthesis of some derivatives of 3-formylrifamycin-SV as potential polymerase inhibitors. Starting with the 3-formylrifamycin-SV, a variety of functional moieties has been added to the 3-position. Considerations were given to such characteristics as chain length, chain branching, position of branching, stereochemistry, hydrophobic and electronic factors. Attachments to the piperazine ring included aliphatic, aromatic and heterocyclic substituents, and replacement of the piperazine ring by bicyclic analogs and higher homologs. These types were prepared and submitted by Dow Chemical Company. Work in this area is continuing.

12. Some of the effort of the Section has been directed towards the synthesis of DNA and receptor site oriented agents. A group of compounds designed to intercalate into DNA in the manner proposed for several antitumor drugs such as actinomycin, daunorubicin, quinacrine and probably ellipticine have been prepared.

A common structural feature has been observed among some non-alkylating anti-leukemic agents. This structural feature consists of a triangulation composed of one nitrogen and two oxygen atoms with rather definite interatomic distances. The preparation of some compounds designed specifically to test this observation has been undertaken and work in this area is continuing at Midwest Research Institute.

One very important advantage of synthesis contracts of this type is that they are placed with laboratories which are able to offer a quick response to a new lead and move quickly into an area of research generated by the Cancer Treatment Program and the various clinical groups. The broad experience and mature judgment in synthetic chemistry of these laboratories place them in a unique position for all types of synthesis. These contractors have provided a great number of compounds with a wide variation in chemical structure.

During the past year, ten new compounds passed Decision Point II, of which nine were synthetic materials. These new synthetic compounds are listed in Table II.

The Chemical and Drug Procurement Section directs 18 contracts for the chemical synthesis of new compounds for the Program (Table VII).

TABLE VII

CONTRACT LABORATORIES FOR SYNTHESIS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Ash-Stevens, Inc.	Ash	NO1-CM-12314
British Columbia, University of	Kutney	NO1-CM-23223
Collaborative Research, Inc.	Friedman	NO1-CM-60506

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Collaborative Research, Inc.	Friedman	N01-CM-12326
Dow Chemical Company	Tong	N01-CM-12313
Dow Chemical Company	McCarthy	N01-CM-33724
Georgia, University of	Blanton/Martinelli	N01-CM-12311
Massachusetts Institute of Technology	Hecht	N01-CM-43712
Michigan, University of	Burckhalter	N01-CM-92004
Microbiological Associates, Inc.	Beisler	N01-CM-33728
Midwest Research Institute	Cheng	N01-CM-33743
Pennwalt Corporation	Block	N01-CM-12309
Rhode Island, University of	Goodman/Abushanab	N01-CM-12312
Sheehan Institute for Research	Razdan	N01-CM-23719
Southern Research Institute	Montgomery	N01-CM-33712
Stanford Research Institute	Henry	N01-CM-33742
Upjohn Company	Martin	N01-CM-23709
Utah, University of	Townsend	N01-CM-23710

Preparative Activities

The resynthesis or preparation laboratories are, in the strictest sense, service laboratories, and are designed and selected to prepare known chemicals and bulk drugs which are needed by the Program. The compounds selected for preparation are not available from the original supplier or on the open market. These laboratories differ from the synthesis contract laboratories in that the synthesis contracts are devoted entirely to the preparation of new compounds while the preparative laboratories are service contracts for the large scale extraction, isolation, and purification of materials from natural sources and for resynthesis of known chemicals.

The preparation laboratories are also used to obtain data for the preparation of the necessary quantities of clinically-important chemicals and to develop the most economical means for their preparation. Usually methods and/or references for the synthesis of the compounds requested are supplied to the contractors by the Section. It should be pointed out that many methods of synthesis which are practical for small quantity are not technically feasible or economically practical when used for a large-scale synthetic operation or for radiolabel synthesis. The conversion of small-scale to large-scale production often requires developmental studies which are conveniently carried out by the preparation laboratories. Solubility and stability studies have also been performed by the preparation laboratories. In addition, some of these contractors have provided material in formulated dose form.

The increased emphasis in clinical investigations has caused a major increase of the preparation activities. An increase in the number of compounds demonstrating confirmed activity will result in an increase in the number of compounds to be resynthesized for completion of screening or confirmational testing. Also, the increased effort being devoted to toxicologic and clinical studies has resulted in the need for even more compounds from this program and in larger quantities.

The preparation laboratories, taken collectively, provide the means of obtaining nearly any type of chemical compound, regardless of structure, and the ability of providing large quantities of very high purity drugs.

1. Bulk chemical and drugs

The compounds chosen for resynthesis at the preparation laboratories are assigned for resynthesis for five major reasons:

(a) For clinical investigations;
(b) For preclinical toxicologic and pharmacologic evaluation;
(c) For maintaining a stock of chemicals of clinical and preclinical interest for study in the Program;
(d) For completion of screening or confirmational testing; and
(e) For use as intermediates in further synthesis.

The compounds so chosen are placed on priority lists based upon their relative importance, with the toxicologic and clinical compounds given top rating. The quantity of a given material to be resynthesized may vary from 1 gram to as much as 200 kilograms. Factors governing the amounts depend upon such considerations as: use, ease of preparation, stability, and cost. Materials of high quality are prepared in the preparation laboratories. Every effort is made to safeguard the clinician in the quality of the drug he uses.

The Chemical and Drug Procurement Section, through the preparative laboratories, directed the resynthesis of 195 compounds totaling 143 kilograms during the calendar year 1973. Included in this group were 57 compounds scheduled for or undergoing clinical evaluation totaling 135 kilograms. In addition to the compounds resynthesized, 152 new compounds were also prepared by the preparative laboratories. During this report period the Section also purchased 15 compounds scheduled for or undergoing clinical evaluation totaling 140 kilograms.

The Section directed eight contract laboratories for the preparation of materials needed by the Program (Table VIII).

TABLE VIII

CONTRACT LABORATORIES FOR PREPARATION OF CLINICAL AND EXPERIMENTAL COMPOUNDS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Aerojet Chemical Co.	Jagow	N01-CM-81300
Aldrich Chemical Co.	Li	N01-CM-23706
Dow Chemical Co.	Collins	N01-CM-12165
Merck and Co.	Babson	N01-CM-22002
Monsanto Research Corporation	Ellard	N01-CM-33709
Parke, Davis and Co.	Elslager	N01-CM-23708
Pharm-Eco	Draper	N01-CM-23707
Starks Associates, Inc.	Starks	N01-CM-23203

2. Radiolabeled Materials

The continued importance of pharmacologic and drug distribution studies has maintained the need for radiolabeled chemicals and drugs. The Section coordinates their procurement and distribution. The required labeled materials are purchased from commercial sources, if available. Materials not available from commercial sources are prepared by contract preparative laboratories. These contracts also handle the storage and distribution and perform the necessary analytical work for all labeled materials, whether prepared under the contracts or acquired from other sources. All requests for radio-active materials are reviewed by a committee in relation to proposed use prior to assignment to the contracts for procurement. During this report period, the Section procured from all sources a total of 20 radiolabeled materials (Table IX) and directed their distribution. One hundred eighteen shipments of radio-active substances were made during calendar year 1973.

TABLE IX

RADIOLABELED MATERIALS PROCURED

NSC NO.	RADIOLABELED MATERIAL	LABEL
740	Methotrexate	H^3(3',5')
3088	Chlorambucil	C^{14}(ring)
13875	Hexamethylmelamine	C^{14}(ring)

NSC NO.	RADIOLABELED MATERIAL	LABEL
19893	Uracil, 5-fluoro-	C^{14}(2)
26271	Cyclophosphamide	C^{14}(ethylene)
63878	Cytosine, 1-B-D-arabinofuranosyl- (ara-c)	C^{14}(2)
79037	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso (CCNU)	C^{14}(ethylene)
79037	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso- (CCNU)	C^{14}(ring)
83265	L-Alanine, 3-(tritylthio)-	C^{14}(amino acid)
83265	L-Alanine, 3-(tritylthio)-	C^{14}(trityl)
85998	Streptozotocin	C^{14}(methyl)
95441	Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso- (Methyl CCNU)	C^{14}(ethylene)
95441	(Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso- (Methyl CCNU)	C^{14}(ring)
102816	5-Azacytidine	C^{14}(4)
104801	Acrylic acid, 3-(*p*-anisoyl)-3-bromo-, sodium salt (Cytembena)	C^{14}(carbonyl and carboxyl)
126849	3-Deazauridine	C^{14}(1')
143647	DL-Alanosine	C^{14}(1)
145668	Cyclocytidine monohydrochloride	C^{14}(2)
154890	Coralyne Sulfoacetate	C^{14}
404241	Adenine, 9-B-*D*-arabinofuranosyl- (Ara-A)	H^{3}(2)

The Section directed two contracts devoted to the procurement of radiolabeled materials for use in preclinical pharmacologic and clinical studies (Table [illegible]).

TABLE X

CONTRACT LABORATORIES FOR PREPARATION OF RADIOLABELED MATERIALS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Monsanto Research Corporation	Yanko	NO1-CM-23715
Stanford Research Institute	Tanabe/Leaffer	NO1-CM-12162

Quality Control

The Branch is keenly aware of the need for providing bulk chemicals and formulated drugs of highest quality for use in clinical investigations and preclinical evaluations. The purpose of quality control, based on rigorous chemical analytical methods, is to evaluate these materials as to their identity, assay and purity. All lots of bulk materials to be used in toxicologic and pharmacologic or in clinical studies are sent for analysis before the materials are released for use or formulation. Also, quality control data is obtained for all formulated materials prior to their release to the clinical investigators.

It should be pointed out that the Cancer Treatment Program deals with a great diversity of chemical compounds, many of which are entirely new with no methods of assay available. Very few compounds are assayed on a routine basis; thus, in many cases, methods of assay must be developed. These assay methods are made available and serve as an invaluable tool to the investigator using the particular compound. Also, the program on analytical studies of anticancer compounds has become involved in working out analytical methods for many of the compounds in biological media such as blood and urine.

Among the techniques that are most commonly used in the drug analysis work are: spectroscopic determinations (ultraviolet, visible, infrared and nuclear magnetic resonance), chromatographic determinations (paper, thin layer, high pressure liquid, and gas-liquid) and titrimetric determinations (aqueous and potentiometric). In addition to the above, elemental microanalysis is carried out. Where applicable, optical rotation, optical rotatory dispersions, refractive index, functional group assay, the degree of hydration or solvolysis determinations and the preparation of derivatives are performed. Chromatographic procedures are most useful in the determination of the presence of a possible unidentified impurity. The combination of this procedure with the melting point, infrared and ultraviolet spectra is often sufficient for the analysis of a simple, stable, well-characterized compound. The determination of the purity of a more complex compound is often an analytical research problem requiring the use of a combination of sophisticated techniques and the consideration of the stability of the compound and its possible contaminants or decomposition products.

In addition to the identification and analysis of compounds under study, the analytical unit is deeply concerned with those physical and chemical characteristics of the compounds which must be considered in the handling, storage, formulation and administration of the material. Of increasing importance to the Program is solubility and stability data, which serves as a guide for drug formulation, storage, and shipping. In certain situations the decomposition of a compound, its solution or formulation may be followed by the observation of the ultraviolet absorption when the decomposition of the active moiety results in a change in absorption. For the stability of compounds which cannot be followed by this procedure, other methods must be devised. Among the other procedures used are chromatography, determination of halide iron concentration, nuclear magnetic resonance, and mass spectrometry. To validate the accuracy of these procedures, determinations are made on a sample to which known standards have been added. The analytical data on compounds of current interest is periodically reviewed and the data is assembled in the form of a Chemical Information Sheet. These Chemical Information Sheets are made available to the investigators using the material.

The need for the evaluation of the "shelf-life" of formulated drugs is an increasing problem. In order to establish the effective life of a drug, the continuing assay of these materials is required and is performed on a regular basis.

In addition, the necessary analytical data for the Food and Drug Administration are collected and assembled. Files are maintained on the drugs in clinical trial in compliance with the Food and Drug Administration regulations covering "Investigational New Drug Applications."

The Unit collects and files such data as:

1. The detailed method of preparation;

2. The supplier's analytical data sheet; and

3. The independent analytical evaluation of each lot of chemical or drug as evaluated by the quality control contracts.

The assembled data is of use in writing purchase specifications for additional quantities of the compound. Attempts are made to keep specifications brief by listing those requirements which are judged to best identify and assay the compounds.

Another function of the quality control group is the analytical evaluation and/or certification of materials purchased on the open market. Inquiries received from scientific investigators and participating clinicians as to drug quality are also handled.

During the calendar year 1973, 185 compounds, representing 355 lots of bulk chemicals, and 152 lots of clinical formulations have been evaluated. Eac of these evaluations is treated individually, and the extent of analysis for each sample is a matter that is jointly decided by the Drug Development Team and the contractors. The analytical data occasionally indicated unacceptable materials resulting in their being returned to the manufacturer for reprocessing.

In addition, several special studies were performed. One analytical facility, Midwest Research Institute, determines the partition coefficient of compounds for use in structure-activity correlation studies. This often involves intensive analytical development in cases where the compound is relatively insoluble or highly soluble in one of the solvents.

Assistance was also extended to the formulation facilities and the preparation laboratories with their analytical and formulation problems concerning compounds of interest to the Program.

The Section directed two analytical-quality control contract laboratories (Table XI). In addition to the two analytical laboratories, elemental analyses are obtained from Micro-Analysis, Inc., under a purchase order agreement.

TABLE XI

CONTRACT LABORATORIES FOR ANALYTICAL-QUALITY CONTROL

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Midwest Research Institute	Haggerty	N01-CM-33722
Stanford Research Institute	Lim	N01-CM-33723

Miscellaneous

One contract, Pomona College is for structure-activity studies. This contract is directed towards (1) the use of the regression analysis techniques developed by Dr. C. Hansch of Pomona College to develop structure-activity correlations of antitumor agents and (2) the investigation of the factors which lead to the parabolic structure-activity relationship in enzyme-substrate systems which will be used to improve the quality of structure-activity predictions for drug-enzyme systems. The Contractor has worked with some of the synthetic contractors to develop quantitive structure activity relationships (QSAR). A structure activity correlation was made for a series of nitrosoureas using Lewis Lung screening data and the optimium lipophilic character for this series of compounds was established. In addition, the Contractor assisted in determining the compounds to be synthesized to develop a lead in order that a reasonable range of lipophilic and electronic properties of the substituents will be obtained and that a regression analysis can be made when the biological data becomes available. Work in these areas is continuing.

The comprehensive investigation of the parabolic structure-activity relationship in enzyme-substrate systems has continued.

TABLE XII

MISCELLANEOUS CONTRACT LABORATORIES

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Pomona College	Smith	N01-CM-33703

NATURAL PRODUCTS SECTION
DRUG DEVELOPMENT BRANCH

Scope

This Section has as its primary objective the development of novel cancer chemotherapeutic agents from higher plants, animals, and microbial sources. This area will be discussed under the headings of Plant and Animal Products Program and Fermentation Products Program.

Staff

This Section has operated with a staff of four persons, consisting of two professional (Ph.D. level) and two clerical employees. Dr. John Douros is responsible for the Fermentation Products Program.

Surveillance

World-wide surveillance of the published literature and collection of chemicals and drugs for biological evaluation are carried out in the Fermentation Products area. With the discontinuance of the formal plant literature survey project and the inability to assign personnel to this work, it has not been possible to undertake surveillance in the Plant and Animal Products area to an appreciable extent. The good results obtained in the fermentation area speak favorably for extension to the plant and animal area.

Accomplishments

The Natural Products Section continues to contribute an increasing number of compounds to the program, many of them of novel structure not readily synthesizable. Those with the highest activity were moved along to confirmation and testing in the slow-growing tumors, B16 melanoma and Lewis lung carcinoma. A substantial number, when large-scale isolation provides more material, will be candidates for Decision Point II.

Table I lists the materials of natural origin acquired during the year under review compared with the previous year. Of the crude extracts, contractors provided 85% of the 2550 fermentation products, 48% of the 2689 animal products and 82% of the 11,661 plant products. A total of 291 purified (mostly crystalline) natural products were received or isolated. About 70 were active and 24 passed the criteria for confirmed activity (Step III). One new compound was assigned to Decision Point IIA. Three compounds were assigned IND numbers, of which two were fermentation products and one was a plant product.

Steps have been taken to make more selective the plant collection program resulting in a greater yield of active plants from a smaller total number, thus reducing the screening costs. The experiment begun last year to improve the plant extraction procedure and give higher yields of actives shows every evidence of success. Changes in the fermentation program are expected to result in higher yields of active agents. All these improvements are outlined in more detail in the appropriate following paragraphs.

PLANT AND ANIMAL PRODUCTS PROGRAM

In carrying out its objectives in the plant and animal products area the Section has, in addition to the contract effort, developed programs with collaborating research grantees and other organizations in (a) plant and animal collecting; (b) preparation of extracts; (c) fractionation of active extracts, and the isolation and identification of the active agents therefrom; and (d) in collaboration with the Chemical and Drug Produrement Section, the preparation of large amounts of active agents from plant materials for preclinical toxicology and clinical trial.

Through extensive correspondence, contact is maintained with potential domestic and foreign suppliers in order to acquire plants, animals, and pure compounds of natural origin which are of special interest to the Program. In addition, this correspondence provides information relating to the use of plants and plant products in medicine (popular, native, and folklore).

The following contractors are engaged in the Plant and Animal Products Program (Table XIII).

In addition, an open-end, fixed-price contract for the procurement of plant extracts has been continued with the University of Costa Rica (Dr. J. Saenz R.) and a similar one for the procurement of marine animal extracts has been continued with the University of Oklahoma (Dr. A. J. Weinheimer).

Table XIII

PLANT AND ANIMAL PRODUCTS CONTRACTORS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Agriculture U.S. Department of	Perdue	Y01-CM-40001 (Transfer)
Arizona, University of	Cole	N01-CM-33750
Arizona State University	Pettit	N01-CM-12308
Central Drug Res. Inst.	Dhar	01-004-1 (PL-480)
Hawaii, University of	Sagawa	N01-CM-33747
Illinois, University of	Farnsworth	N01-CM-22078
Research Triangle Inst.	Wall	N01-CM-92019
Virginia, University of	Kupchan	N01-CM-12099
WARF Institute, Inc.	Coon	N01-CM-43707

Beside the contractors listed above, several groups, including grantees and those not supported by the NIH, contribute in different ways to this program without cost to DR&D. The major groups are: C.S.I.R.O. (Australia),

U.S.D.A. (Pesticide Chemical Research Branch, Beltsville), U.S.D.A. (NURDD, Peoria), C.S.I.R. (South Africa), National Defense Medical Center (Taiwan), Purdue University (Dr. Cassady), Virginia Polytechnic Institute (Dr. Kingston), and Univ. de Concepcion, Chile (Dr. Silva).

Progress was made in the following areas of plant and animal products:

A. Marine organisms

During the year, 2689 animal products, mostly marine, were accessioned. About 180 different species are active in Leukemia P388 (PS) and 22 species in KB cell culture, and about 72 species are currently under fractionation. This has proved to be an unusually difficult field chemically, due probably to the nature of the active agents and the accompanying impurities. Highly active (in PS) fractions are being obtained, however, and it is anticipated that pure products will be forthcoming from this work in the near future.

B. Plants

1. Nomenclature review. Good progress has been made by the U.S.D.A. in reviewing the 73,000-odd botanical names in our plant accessions to assure technically correct Latin names. This will (1) eliminate synonymy, thereby avoiding future conflicts where the same plant under two different names is being fractionated by two chemists, (2) bring the nomenclature in line with other large collections, including that of the U.S.D.A., thus making computer analyses of data more significant, and (3) provide accurate names for the future publications of the fractionators and others using the file.

2. Program Review. This is a joint project of the U.S.D.A. and the Section to review the total plant input and the chemical output of the program for the purpose of recognizing plant families that (a) are known to produce compounds of interest to our program or that have been inadequately screened, in either case making them deserving of special effort in collecting, and (b) are known not to produce compounds of interest to our program, or that have been adequately screened, in either case making them subject to avoidance in collection.

   This selective collection has been in effect for some time and is already yielding promising results. Plants in category (a), known to produce compounds of interest, are being collected in large quantities suitable for extraction without the necessity for recollection, and already the indications are that a substantially higher yield of confirmed actives is being obtained. Plants in category (a), that have been inadequately screened, are being sought successfully by planned collecting in countries known phytogeographically to be rich in such families. Such collections are yielding a substantial number of confirmed actives.

   Thus, by this means, a rational approach to plant collecting has been developed, combining guidance provided by chemical feedback with a modified random collecting necessary to explore new areas. Such an approach could not have been considered until our data had become as extensive as it is, and it is anticipated that in the future our collecting will be substantially more efficient and responsive to the needs of the program.

3. Duplicate Searching. Following the establishment of a computer "terminal" at the U.S.D.A., it is possible for the U.S.D.A. to communicate telephonically with our plant genus and species "bank" and instantly determine duplicates among accessions throughout the year. Such duplicates are not sent out for extraction and screening, and this represents not only a great saving in the expense of extracting and screening these plants but saves considerable time on the part of our own staff.

4. Large Re-collections. The following large re-collections needed for the preparation of clinical quantities of drugs or of relatively large amounts of drugs for advanced testing were made during the year under review: Brucea antidysenterica stembark (for bruceantin, NSC-165,563), 5000 lbs. from Ethiopia; Thalictrum dasycarpum seed (for thalicarpine, NSC-68075), 6400 lbs. from Minnesota, Wisconsin, Michigan and southern Ontario; and Bouvardia ternifolia, whole plant (for compound B613763 F049), 2000 lbs. from Mexico.

5. Improvements in the Plant Extraction Procedure. Following discussions at the November 1971 Annapolis contractors' meeting and decisions made thereafter, it has been possible to implement a recommendation directed toward increasing the yield of LE-actives by subjecting 500 plant materials to a procedure designed to concentrate any LE-activity present to a level where it can be detected by the regular screen. This procedure is a preliminary fractionation by solvent partition yielding five extracts from every plant material. Each extract is submitted to the regular PS and KB screens and those that show confirmed activity in either of these tumors are submitted to testing in LE. A large enough number of confirmed actives in PS and KB have appeared already to suggest that two of the five fractions each gives a higher yield of confirmed actives than does the standard extraction method. This experiment is being closely monitored. If this result is maintained it will call for a revision of our procedure for working up plant material with the possibility that fewer plants can be used to obtain an adequate input of actives for fractionation.

6. Decision Network IIA and IND Materials. One plant-derived compound - coralyne sulfoacetate (NSC-154,890) - passed DN IIA, and another - d-tetrandrine (NSC-77037) was the subject of an IND filing. These small numbers reflect (a) the replacement of the Walker 256 tumor (WA) by Leukemia P388 (PS) in the general screen with the resulting necessity to build up the "pipeline" of new active isolates, (b) certain difficulties of scale-up procedures of preparation and scheduling due to small quantities of active agents present in the plant materials, and (c) certain difficulties with some of the formulations. There is no shortage of candidate compounds arriving at the point of advanced screening.

FERMENTATION PRODUCTS PROGRAM

Since the initiation of antibiotic research and fermentation in the early 1940's, microorganisms have been an extremely important source of new chemotherapeutic agents in the infectious disease field and 40 have been evaluated in U.S. clinical studies for antineoplastic activity. Antibiotics have a great potential in cancer chemotherapy as primary treatment agents or as the initial structural building blocks for new and novel compounds that can be synthesized and used in the clinic.

The present fermentation program has been carried out through contracts at different biological laboratories listed in Table XIV:

Table XIV

FERMENTATION CONTRACTORS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Bristol Laboratories	Bradner	N01-CM-90035
Bristol Laboratories	Claridge	N01-CM-43759
Georgetown University	Katz	N01-CM-43732
Illinois, University of	Rinehart	N01-CM-23208
Litton Bionetics (Project 17)	Perritt	N01-CM-23294
Parke-Davis & Co.	Dion	N01-CM-43754
Upjohn Co.	Neil	N01-CM-43753

Of these, four have been in effect less than one year: Bristol Laboratories (N01-CM-43759), Parke-Davis & Co., Litton Bionetics (Project 17) and Georgetown University.

A computer analysis of the activity in PS of all the microbial cultures tested in our program revealed an unexpectedly large number of highly active ones (Table XV):

Table XV

NUMBER OF CULTURES ACTIVE IN PS

NAME OF ORGANIZATION	T/C 125-149	T/C 150-174	T/C 175-199	T/C >200	TOTAL
Michigan Health	86	104	37	20	247
W. R. Grace	39	22	7	5	73
Bristol	4	8	1	2	15
Upjohn	16	29	15	18	78
Pfizer	2	3	3	3	11
TOTAL	147	166	63	48	424

Thus, there is a total of 111 cultures with activities in PS of 175% or higher which are candidates for fractionation. Of these, twenty-four culture fermentations are under chemical fractionation by either the fermentation contractors or the contract plant fractionators, and it is expected that at least five will be purified and evaluated in the coming year. Several of the high priority culture fermentations have been assigned to plant fractionators for isolation of the active agents in order to reduce our backlog with the fermentation contractors and to take advantage of the expertise of the plant fractionators. Additional antibiotics will continually be obtained from our surveillance program and will be evaluated.

Surveillance

World-wide surveillance of the published literature and collection of purified products for biological evaluation are carried out in the fermentation products area. Two-hundred and six inquiries were made to companies and universities for materials to be tested. This output yielded 136 compounds for testing.

Statistical Summary

Table I lists the natural product materials acquired during the fiscal year 1973 compared with previous years. Of the crudes, contractors provided approximately 85% of the 2,550 fermentation products. Of these, 267 were active in at least one of our test systems. Of the 136 purified or crystalline materials received, 5 (or 4%) were from our contractors. IND's were filed on 2 products: methanol extraction residue (MER) of BCG (NSC-143,769) and BCG (Tice strain) (NSC-116,327). Forty antibiotics have been in clinical study by DCT, and up to the present time 14 are still under clinical investigation. The following two additional drugs have recently been scheduled for Phase I clinical studies - Neocarzinostatin and Anguidine. Several other antibiotics are being clinically evaluated in other parts of the world.

They are Carminomycin, Olivomycin A, Acetyl kidamycin, Leucinostatin, and Actinomycin C. All but Actinomycin C are under development in our program. Renewed interest in the actinomycins has resulted from new information due to DNA-complexing properties and solid tumor data.

Project 17.

NCI has initiated a fermentation project at the Frederick Cancer Research Center, Frederick, Md., to take advantage of existing fermentation and analytical facilities to investigate the full potential of fermentation in the cancer chemotherapy field.

Project 17 has two major objectives:

1. To produce, scale-up and develop in pilot plant quantities, antineoplastic antibiotics which are obtained from several of our present contracts and from our own research at FCRC. Limited pilot plant capacity has been a major drawback in our present contract fermentation program. Materials needed for chemical purification, advanced testing etc. have not been more rapidly forthcoming because of direct competition for facilities with the pharmaceutical companies' own research program.

2. To evaluate several areas of microbial fermentation which have been investigated no more than partially or not at all in our previous NCI fermentation contract programs such as:

    a. Microbial biotransformations of antibiotics, plant materials and synthetic chemicals which have demonstrated limited activity, toxicity, solubility problems, etc. It is anticipated that these problems may be overcome by the use of this technique.

    b. Evaluation of thermophilic, psychrophilic, osmophilic and halophilic organisms for their ability to produce antineoplastic antibiotics.

    c. Utilization of unusual substrates to obtain bizarre organisms and then ferment routinely.

    d. Co-metabolism of (a) and (c).

    e. Continuous fermentation of known producers of antineoplastic antibiotics, to reduce the costs of the process and to select better strains of organisms.

    f. Mixed culture fermentation of various antineoplastic-producing organisms to produce novel materials.

    g. The use of techniques of mutation, strain selection, stimulants and enzyme inhibitors to change the metabolism of organisms in order to produce more or different antibiotics.

Several of our present fermentation contracts have as facets of their programs certain of the above-mentioned research subjects. With the input from Project 17 these areas should yield within the next several years some highly active and interesting antibiotics.

Plans for the fermentation pilot plant and microbiological research laboratories have been completed. The construction of the laboratory will be initiated in early 1975 and it is anticipated that both laboratories and pilot plant will be operational during FY 1975. A staff of 18-20 persons is expected on Project 17.

Future Plans

Three groups of organisms have been shown to be the principal producers of antibiotics; they are bacteria, fungi, and actinomycetes. There are still multitudes of new and different organisms to be isolated using unusual substrates, radical environmental conditions and various unique soils and isolation techniques. In addition, various fermentation and genetic techniques should stimulate the production of more and unique antineoplastic antibiotics. Two of these techniques have been introduced into our contract fermentation programs: co-metabolism and biotransformation. Their possibilities are limited only by the ingenuity of the microbiologists.

There are several areas of fermentation that have not been exploited for their ability to produce new antineoplastic materials. Among these are (a) the use of a variety of different carbon sources for fermentation substrates and as co-oxidizing materials; (b) microbial transformations of the known toxic, active and inactive antibiotics and other natural products, as well as synthetic compounds, to determine if microbial modification can convert them into clinically useful drugs; and (c) the study of yeasts, of thermophilic and psychrophilic microbes, and of mixed cultures.

DRUG DESIGN AND CHEMISTRY SECTION
DRUG DEVELOPMENT BRANCH

SCOPE

The Drug Design and Chemistry Section is primarily responsible for the intramural research of the Drug Development Branch. The objectives of this work are: (1) chemical research in the design and synthesis of new drugs for the treatment of cancer, (2) the chemistry of agents and drugs of interest in the treatment of cancer, (3) investigation of the chemistry of natural products as potential antitumor drugs, (4) research on the structure, purity, decomposition products and metabolites of antitumor drugs by analytical instrumental techniques, and (5) the development of structure-activity techniques to summarize existing data and to provide guidelines for analog synthesis.

STAFF

The staff consists of seven professional scientists and a secretary. Most of the professional staff is composed of N.I.H. Visiting Fellows and N.I.H. Staff Fellows. In addition, several persons are employed on the College Work-Study Program as part-time technicians.

SUMMARY OF ACCOMPLISHMENTS

Approximately six man-years effort was devoted to the intramural chemical research projects described in Individual Project Reports 3580 and 3581. Areas of synthetic organic interest include central nervous system antitumor drugs (hydantoins, phenothiazines, quinones and procarbazine analogs) and amino sugar derivatives. Analytical research centers on nuclear magnetic resonance microtechniques. Structure-antitumor activity studies were carried out for quinones, psychotropic drugs and rifamycin derivatives. The most significant accomplishments include the synthesis of a new aziridinyl quinone with confirmed L1210 activity of T/C $\gg$200%. This compound should have an excellent chance of having significant intracerebral (IC) antitumor activity. A series of hydantoin mustards was synthesized as potential CNS antitumor agents. At least one new compound in this series has activity in a broad spectrum of tumor systems (L1210, B16, LL, P388 and ependymoblastomia). NMR techniques were used to develop a method for studying drug and impurity binding to L-asparaginase. A structure-activity study covering 1500 quinones tested in the DR&D program was completed.

Drug Design & Chemistry personnel were responsible for planning two symposia devoted to "Structure Activity Techniques in the Treatment of Antitumor Drug Data" and "Quinones as Anticancer Agents". They delivered presentations at both these meetings as well as one describing adriamycin-like antibiotics at the Adriamycin Symposium.

Section personnel were involved with contract planning and/or competition in five organic synthetic research areas. They were project officers on three research contracts.

Serial No. NCI 3580
1. DCT, DR&D
2. Drug Development Branch
3. Drug Design and Chemistry Section
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Chemical Research in the Development of New Anticancer Drugs

Previous Serial Number: NCI 3580

Principal Investigator: John S. Driscoll

Other Investigators: T. Hirata, A. H. Khan, S. Chu, B-L. Chu, G. Peng, T. Krishnamurthy, H. B. Wood, Jr.

Man-Years: Total: 4.4
Professional: 4.4

Project Description:

The objective of this project is the discovery of new types of clinically useful anticancer drugs. Organic synthesis research is directed towards the preparation of new compounds which have potential as useful agents. Leads for this program are generated from structure-activity studies, the random screening program and the literature. Structure-activity studies are carried out on classes of compounds with indications of antitumor potential. Computer techniques are utilized in the study of quantitative relationships among congeners, and in the analysis of the large masses of screening data associated with qualitative structure-activity studies.

Specific Objectives:

1. The development of CNS active antitumor drugs.

2. Determination of the active component in decomposed NSC 50741.

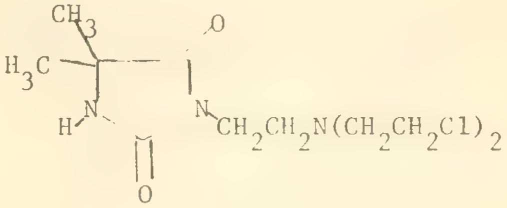
3. Structure-activity studies on quinones, psychotropic drugs and rifamycin derivatives.

Major Findings:

A. CNS Target Directed Antitumor Agents

1. Hydantoins (Dr. Peng)

A literature report that diphenylhydantoin (DPH) concentrates in brain tumors prompted a study of hydantoins with alkylating groups as possible brain and CNS antitumor agents. Since lipophilic character is generally recognized as an important parameter affecting the activity of CNS drugs, six compounds (NSC 152564, 172112-115, 177528) with calculated log P values of ca. 1.5 to 5.0 were prepared. Several of these compounds have log P values similar to that experimentally determined for DPH (log P 2.5). The structure of a typical member of this series (NSC 172113) is shown below.

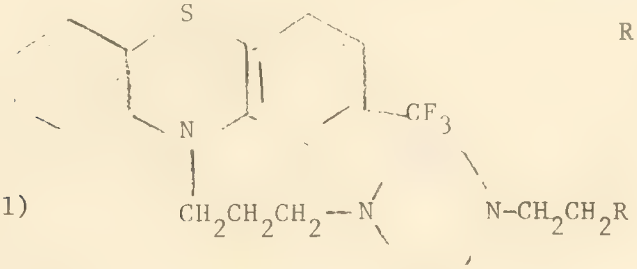


(NSC 172113)

Initial tests were carried out in the standard intraperitoneal (IP) test systems. Selection of candidates for murine intracerebral (IC) antitumor testing is based on the IP results. All six compounds showed IP 388 activity (T/C 200-375%) and IP B16 activity (T/C 145-162%). Four derivatives had IP L1210 activity (T/C 134-141%). The IC L1210 and P388 testing done thus far has resulted in toxic tests. Lower dose testing is underway. Three of these compounds have been tested in the IC ependymoblastomia tumor system. NSC 172113 gave maximum T/C values of 198 and 364% in separate controls. Similarly, NSC 172114 (T/C 370, 158%) and 177528 (T/C 136,148%) were active. Additional tests are in progress.

2. Phenothiazines (Dr. Peng, Dr. Hirata)

A preliminary structure-activity study of the phenothiazines already tested in the DR&D program indicated this class of compounds had antitumor potential. Since the CNS activity of this class of compounds is well established, phenothiazine derivatives appeared to be good candidates as potential CNS antitumor agents. An initial series of 12 specially designed compounds was synthesized and tested. Four additional derivatives were then prepared for an elaboration of steric and electronic effects in the side chain of the molecule. All 16 compounds had the general structure (1).



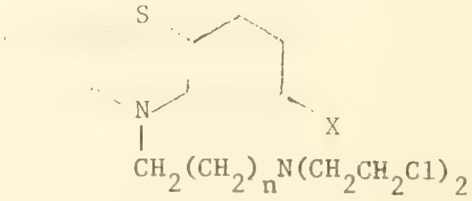
(1)

R = O-alkyl, O-aryl,
NH-alkyl, NHNH-alkyl

Seven of these compounds (NSC 167585, 169673, 169674, 169675, 169676, 171782, 174482) were active in at least one DR&D screening system. Four of the seven were L1210 active (T/C 126-151%), three were KB active and two had low level (T/C 126%) B16 melanoma activity. The L1210 actives were derivatives of (1) where R = oxygen attachment. When R = nitrogen attachment, the compounds were very toxic and these materials made up the KB actives. The IP L1210 actives were also scheduled for IC L1210 testing. No IC activity has been observed yet, but useful test data has been obtained for only a few of these materials.

The test results are now almost complete on a second series of phenothiazines synthesized as carriers for an alkylating group (2).

(2)



$CH_2(CH_2)_nN(CH_2CH_2Cl)_2$

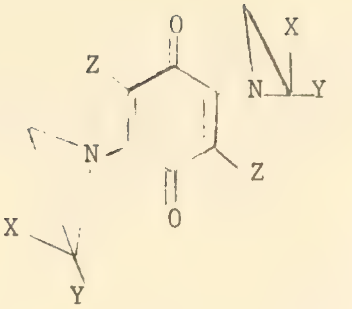
$X = H, CF_3, C_2H_5, Cl$

$n = 1,2$

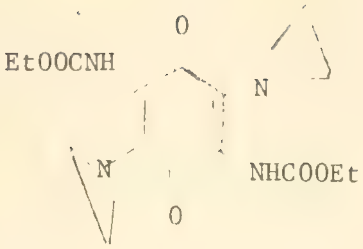
Earlier tests showed that NSC 57694 [(Compound 2(R = H, n = 1)] had good P388 activity (T/C 200%). In the phenothiazine series used as tranquilizers, optimum activity is associated with n = 2 and R = an electron withdrawing substituent, e.g. Cl. The compounds of general structure (2) were prepared to see if the tranquilizer structure-activity relationship was also characteristic of antitumor activity. All four of the compounds prepared (NSC 49922, 166011, 166184, 166185) had some type of activity (L1210, P388, B16, KB) but the level of P388 activity (T/C 130-150%) does not approach that of the parent compound (NSC 57694).

3. Aziridinyl Quinones (Dr. Khan)

Structure-antitumor activity analyses for quinones in general and aziridinyl quinones in particular (described in Section C.1. of this Project Report) indicated that certain aziridinyl quinones were good candidates for exploration as CNS antitumor agents. Major objectives of this project are (1) the development of a compound with improved solubility characteristics relative to the compounds already tested (2) retention or improvement of IP L1210 activity and (3) the synthesis of a compound which satisfies objectives 1 and 2 and which possesses intracerebral L1210 activity. Twenty-three derivatives have been synthesized and submitted for IP L1210 testing to date. Most of these compounds have general structure (3).



(3)



NSC 182986

$X = H, CH_3$

$Y = H, CH_3$

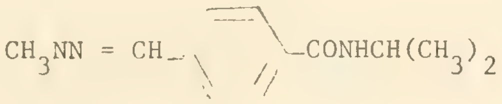
$Z = F, Cl, Br, NR_2, SR$

The screening results on the first 10 derivatives indicate that NSC 182986 shows initial promise. This compound has shown confirmed activity of T/C = 201% in the IP L1210 system.

The initial results also indicate the monomethylaziridinal analog of NSC 182986 has L1210 activity (T/C 151%). NSC 182986 is scheduled for IC L1210 testing as well as evaluation in other significant test systems. A better evaluation of the final series of derivatives to be synthesized can be made when test data are available for all the compounds synthesized thus far.

4. Procarbazine Analogs (Dr. Krishnamurthy, Dr. B-L. Chu)

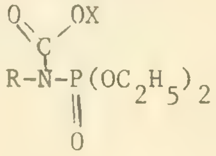
Procarbazine is a clinically useful anticancer drug which enters the CNS. Very few analogs of this compound have been tested in the DR&D program. A study of the structure-activity relationships in this series was recently initiated. The first compound prepared was the hydrazone (NSC 208646), an oxidation product of procarbazine. Test results are not yet available. Current emphasis is on the effect of isomers, isosters and derivatives of the methylhydrazine group.



NSC 208646

5. Phosphorous Compounds (Dr. S. Chu)

One possible mechanism for explaining nitrosourea activity and/or toxicity is the generation of isocyanates inside a tumor cell after transport of the drug in the form of the less reactive nitrosourea. Other types of pro-drugs or "masked" drugs could have significant usefulness to the DR&D program. Certain phosphoramidate derivatives (4) are known to decompose to isocyanates. No examples of these materials had been previously evaluated in our program. Ten members of the general series (4) were synthesized. Two of these compounds (NSC 183766, 183767) had marginal L1210 activity (T/C 128 - 125%). Additional testing is in progress.

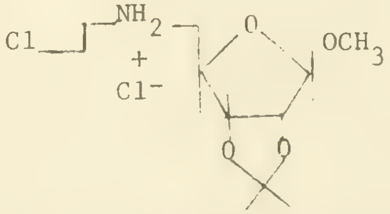


R = alkyl, cycloalkyl

X = Na, C_2H_5

(4)

B. Determination of the Active Component in Decomposed NSC 50741



NSC 50741

NSC 50741 is a L1210 inactive compound which decomposed upon standing for 5 years at 4° to give a L1210 active mixture (T/C 200%). During the past several years, experimental procedures have been developed to shorten to 5 hours the time required to produce an active mixture. The objectives of the current work are (a) the reproducible production of the active mixture under optimal conditions and (b) the isolation, identification and synthesis of the active component.

1. Degradation Studies (Dr. B-L. Chu)

The standard conditions previously established for production of the active mixture involve heating NSC 50741 in aqueous solution (100 mg/ml) at 60° ± 0.5° for 5.0 hours. Several other decomposition techniques were recently studied in order to optimize conditions and gain insight into the mechanism of the decomposition.

a. Acid hydrolysis

A nuclear magnetic resonance (nmr) study utilizing 1N DCl in D_2O as solvent established that a crucial part of the decomposition reaction is finished in 97 minutes at 24°. Four tests of this decomposition method gave a L1210 active mixture (T/C 150-200%) in each case.

b. Water concentration

In an attempt to more clearly approximate the original 5 year, 4° decomposition conditions, the compound to water ratio was increased 50 times relative to our standard conditions. At 60°, a dark syrup was formed which was very difficult to lyophilize but which was L1210 active (T/C 151-187%) in three tests. Chromatography on charcoal abolished activity. Removing water completely, i.e. 60° in a dry flask for 5 hours, resulted in no change in the NSC 50741 and no activity produced. A purely thermal reaction mechanism is, therefore, unlikely.

c. Organic solvents

In order to further study thermal effects and the water requirements for the production of active material, several organic solvents in which NSC 50741 is soluble were substituted for water in 60°, 5 hour experiments.

(1) Anhydrous ethanol and 95% ethanol both neat and with 2% water added produced inactive material.

(2) Chloroform neat gave an inactive product but chloroform with 2% water gave active material with the activity concentrated in the aqueous phase.

These results again substantiate the conclusion that a simple thermal reaction is not involved. The inability of the protic solvent ethanol to produce active material may be related to the alcohol's ability to associate with water which appears to be necessary for activity.

d. Effect of reducing agents

Earlier data indicated that there was an increase in activity if oxygen was removed from the aqueous solution before heating. A reduced dihydropyridine might be an active component. As a test of this possibility, sodium dithionite was added to the reaction. A toxic but inactive mixture was produced.

2. Attempts to Isolate the Active Component (Dr. B-L. Chu)

Dry column chromatography of the acid hydrolysate on cellulose produced one L1210 active fraction (T/C 150%) which was still a mixture. This fraction was the most polar of the five fractions isolated. Chromatography of a mixture on silica gel produced a stable, inactive, white crystalline material. Although the structure of this compound is not yet known, it appears to be a pyridine rearrangement product.

Because NSC 50741 is soluble in chloroform, but the more polar decomposition products might not be, a sample of decomposed material was extracted with chloroform. While the resulting product was active, no activity enhancement took place.

3. Special Biological Testing (Dr. B-L. Chu)

Since the decomposition of NSC 50741 to an active mixture is accelerated by HCl, the compound was administered orally to mice with L1210 leukemia in the expectation that the active component might be produced in the rodent stomach. No activity was observed. A number of L1210 *in vivo* active samples were tested in the KB cell culture system. No activity was observed *in vitro*.

C. Structure-Activity Studies

1. Quinone Study (Dr. Driscoll, Mr. Hazard, Dr. Wood, Dr. Goldin)

A retrospective analysis of the structure-antitumor activity relationships among 1500 quinones screened in the DR&D program was completed. It will appear as the April 1974 issue of *Cancer Chemotherapy Reports*. Natural products such as Mitomycin C, Adriamycin, Daunomycin, Streptonigrin, Lapachol and their derivatives made up the majority of active compounds. Among the synthetic compounds tested, aziridinyl quinones comprise the most L1210 active family. A one day symposium "Quinones as Anticancer Agents" was held on December 7, 1973, as a result of this investigation. Over 50 people attended to hear a summary of the results of the study as well as a discussion of more specific quinone-antitumor topics by five invited speakers.

2. CNS Quinone Study (Dr. Driscoll, Dr. Geran)

One result of the general quinone study described above was the recognition that aziridinyl quinones might have the proper physical and chemical properties to pass the blood-brain-barrier and have CNS antitumor properties. IC L1210 tests were scheduled to test this hypothesis and the results were very encouraging. This led to (a) the initiation of an intramural research project (A.3. this report) to improve upon the properties of the compounds already available, and (b) a more thorough examination of the aziridinyl quinones in several intracerebral (IC) systems

(IC ependymoblastomia, IC L1210, IC P388). Significant activity (T/C 150-300%) has been observed in these IC systems. These leads are being further pursued through additional screening and an intramural synthesis project.

3. Psychotropic Drug Study (Dr. Driscoll, Ms. Lomax and Melnick, Mr. Quinn, Ing and Davignon)

   Over 250 psychotropic drugs (tranquilizers, psychoenergizers, etc.) were obtained from 64 U. S. and foreign pharmaceutical companies for (a) general antitumor testing and (b) the generation of leads for CNS antitumor agents. This group of compounds contains many structural classes new to our program. Screening results are almost complete. Twenty-nine compounds have shown at least minimal activity (T/C >125%) in the IP L1210 leukemia and/or the B16 melanoma systems. Phenothiazines, their isosters and β-phenethylamine derivatives are prominent among the actives. Further biological studies are underway with the active compounds.

4. Rifamycin Study (Mr. Quinn, Dr. Driscoll)

   A manuscript describing a Hansch-type structure-antibacterial activity correlation obtained from literature data for 75 rifamycin derivatives has been prepared.

PUBLICATIONS

Hirata, T., Wood, H. B., and Driscoll, J. S.: Rearrangement and Elimination Reactions in 1,2,4-Triazole Derivatives. J. Chem. Soc. (London), 1209, 1973.

Driscoll, J. S., Hazard, G. M., Wood, H. B. and Goldin, A.: Structure-Antitumor Activity Relationships Among Quinone Derivatives. Cancer Chemother. Reports, Part 2, in press.

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1. DCT, DR&D
2. Drug Development Branch
3. Drug Design and Chemistry Section
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Analytical Chemistry of New Anticancer Drugs

Previous Serial Number: NCI 3581

Principal Investigator: J. S. Driscoll

Other Investigators: Li-Ming Twanmoh, Ilga Higbee and Harry B. Wood, Jr.

Man Years: Total - 1.5
Professional - 1.5

Project Description:

General Objectives:

The objective of this project is the research and development of analytical techniques which are used to:

1. establish the structure and purity of new anticancer drug candidates;

2. establish the identity and purity of bulk and formulated drugs;

3. determine important physical and chemical properties of new anticancer drugs; and

4. study reaction mechanisms and structure-activity relationships.

Method Employed:

Emphasis is placed on modern instrumental techniques. Primary emphasis is placed on the use of high resolution nmr spectroscopy and glc as analytical tools. Chromatographic techniques, as well as infrared and ultraviolet spectroscopy, are also extensively employed.

Major Findings:

A. Nmr Microtechniques (Dr. Twanmoh)

Nmr spectroscopy is a non-destructive structural elucidation technique which usually requires about 25 milligrams of material. However, this may be much more than is available for some biological samples.

In addition, if the effluent from an analytical gas-liquid chromatograph is to be collected for nmr analysis, 25 mg is far too large a sample size for routine work. The objective of this investigation is the determination of the optimum conditions and the minimum amount of sample required to obtain a useful nmr spectrum using proton Fourier transform nmr spectroscopy, a special technique available in the Drug Development Branch. Microcells had been previously evaluated. Using the optimum microcell, methylene chloride was selected as the best internal standard and experiments were conducted to compare nmr spectroscopy and glc as quantitative analytical techniques for o, p - DDD. The cut-and-weigh technique was employed for producing the quantitative nmr data. The nmr technique, with a relative error of ± 20% in the 0.4 to 3 mg range was less precise than glc. Nmr microtechniques, therefore, appear better suited to structure determination than to quantitative analysis.

B. Cooperative Studies

1. Enzyme-Drug Binding Studies [Drs. Twanmoh, Cooney (NCI-LT), Cohen (NICHD)]

Bound asparatic acid was found as a low level impurity in certain lots of L-asparaginase. Nmr relaxation time studies and equilibrium dialysis measurements are being conducted to elucidate the mechanism of binding. Initial results indicate that the β-carboxyl group participates in the binding process. Studies with other drugs and substrates in the L-asparaginase system are planned.

2. Analysis of CCNU Experimental Formulations (Dr. Higbee, Mr. Cradock)

Emulphor is a surface-active agent used to help solubilize nitrosoureas for parenteral administration. This material complicates the standard nitrosourea UV assay, however. Pyrolysis glc, nmr, non-aqueous redox titrations and solvent extraction/UV analyses were explored as analytical methods for CCNU in the presence of emulphor. The best results were obtained with a cyclohexane extraction of the aqueous CCNU-emulphor solution and UV assay of the hydrocarbon layer for CCNU. Using this assay, it was determined that approximately 5% decomposition occurs over a 4 hour period when CCNU is dissolved in the aqueous emulphor solution. These data correspond well with the results obtained by pyrolysis glc.

3. 6-Hydroxydopamine Assay [Dr. Higbee, Mr. Kleinman (CC)]

The clinical formulation of 6-hydroxydopamine (6-HD) undergoes oxidative decomposition very readily. A spectrophotometric assay was developed using a mixed solvent system (DMSO-THF) which allowed a determination of the rate of decomposition of 6-HD in the clinical formulation.

4. Nmr Spectra Analysis (Dr. Twanmoh)

Short term cooperative studies were carried out with five research groups. Nineteen samples were analyzed.

C. Miscellaneous Studies

1. Nmr Data for Pattern Recognition Studies (Dr. Twanmoh)

The nmr absorption frequencies for seven agents with established antitumor properties (Ara-C 5-FUDR, thioguanine, MTX, CCNU, BCNU and DIC) were determined and analyzed. No obviously common features were characteristic of all compounds. A computer assisted analysis may establish non-obvious patterns.

2. Low Concentration Nmr Studies (Dr. Twanmoh)

In earlier studies, it was shown that Fourier transform nmr spectroscopy could give useful spectra on samples as small as 20 micrograms with certain antitumor drugs (Ara-C, thio-TEPA, diethylstilbesterol). In an attempt to extend this technique to other drugs, adriamycin, daunomycin, vincristine and vinblastine were studied. All of these compounds required much larger amounts (10 to 50X) in order to obtain useful spectral data. This may be related to the structural complexity of the latter group of compounds.

PUBLICATIONS

Twanmoh, L-M., Wood, H.B., and Driscoll, J.S.: Nmr Spectral Characteristics of N-H Protons in Purine Derivatives. J. Heterocyclic Chem. 10: 187, 1973.

CLINICAL DRUG DISTRIBUTION SECTION

DRUG DEVELOPMENT BRANCH

Scope

The Clinical Drug Distribution Section is primarily responsible for the development, production, control, storage and distribution of all investigational dosage forms of interest to the Division of Cancer Treatment Program. The Section is also responsible for the procurement and distribution of commercial formulations used by the Clinical Cooperative Groups. The responsibilities of the Section are as follows:

1. Development of investigational formulations for clinical use;
2. Production and packaging of investigational drugs;
3. Procurement by direct purchase of formulations for clinical use;
4. Quality control surveillance of all clinical dosage forms;
5. Preparation of pharmaceutical data for FDA-IND submissions; and
6. Storage and Distribution of clinical dosage forms.

Staff

The staffing of this Section consists of three Pharmacists, three shipping and receiving clerks and one secretary.

Development of New Dosage Forms for Clinical Use

Formulation development commences when an agent passes Decision Network IIA. The first approach to development involves an evaluation of existing chemical and physical data and a review of *in vivo* route and regimen studies in rodents. The accumulation and evaluation of these data are critical to the ultimate design of the dosage.

Developmental studies are assigned by the Section to one of the contract pharmaceutical laboratories. In preparing the assignment, the contractor is provided with the route of administration, dose level, and available supporting chemical data. Consultation with the Project Officer is carried out concerning feasible formulation approaches.

Routinely, agents considered for parenteral formulation are subjected to solubility and stability studies in a selected group of physiologically accepted vehicles. The drug solutions are then studied for the effects of pH, temperature, light and atmospheric oxygen. Additionally, compatibility tests are performed with buffers, preservatives and vial closures. Agents considered for oral formulation are studied for compression properties, compatibility with binders, photodegradation and temperature effects.

Some of the drugs recommended for development are either insoluble or poorly soluble in most commonly used physiological vehicles. Occasionally, these compounds are highly reactive and require special handling. These problems have been an obstacle in the preparation of some parenteral dosage forms of potential antitumor agents. The contract with the University of Kansas is exploring these formulation difficulties. The contract is designed to perform research on current formulation problems and to design new approaches to parenteral drug delivery.

Experimental formulations are submitted for evaluation and confirmation of antitumor activity. Simultaneously, with these studies, the dosage forms are sent to an independent laboratory for chemical and/or biological analyses.

The toxicological results and the accumulated pharmaceutical development data are distributed to members of the Dose Formulation Committee. These data serve as a basis for determining the starting human dose and the ultimate dosage form to be used in the initial clinical studies. Several dosage strengths of the same investigational drug may be prepared provided the drug concentration remains within the development limits of the dosage form.

During 1973, twenty-five projects for dosage form development were assigned. Seventeen of these involved new drug research and eight assignments were for modification of existing preparations. A total of nine new dosage forms entered clinical trial from the contract efforts, four of which were new drug submissions.

Production and Packaging of Investigational Drugs

An important function of the Section is to provide the Clinical Program with sufficient quantities of formulated materials. The production of dosage forms is mainly carried out by contract agreement with qualified pharmaceutical manufacturers.

The production output of clinical dosage forms for 1973 remained at a high level of effort. The formulation contractors produced a combined total of 40,138 bottles of tablets or capsules (1,255,005 oral doses) and 458,377 vials (parenteral doses). Production output which is dictated by clinical needs has continued to increase during the last few years. The production contractorshave been able to meet the Program's needs for formulated materials.

Three production contracts and one development contract (University of Kansas) (Table XVI) were monitored by this Section.

TABLE XVI
CONTRACTS MONITORED BY THE CLINICAL DRUG DISTRIBUTION SECTION

CONTRACTOR	INVESTIGATOR	CONTRACT NUMBER
Ben Venue Laboratories, Inc.	Wickes	NO1-CM-43701
Kansas, University of	Higuchi	NO1-CM-23217
Philips Roxane Laboratories, Inc.	Marine	NO1-CM-12139
Tennessee, University of	Avis	NO1-CM-02084

Some contractors primarily engaged in bulk drug preparation have capabilities for dosage form production. This Section encourages these contractors to develop and produce their own dosage forms. Specifications for formulation and production are provided to each contractor by this Section. This arrangement has proven economical, expedient and extremely beneficial to the program. Two contractors, The Upjohn Company (Contract NO1-CM-43753) and Bristol Laboratories (Contract NO1-CM-90035), have provided formulated materials by this arrangement during 1973.

Dosage forms are developed and produced by pharmaceutical manufacturers in cooperation with the Division of Cancer Treatment. These sources include several foreign drug manufacturers. The dosage forms are prepared from specifications which are established by mutual agreement between this Section and the manufacturer. During 1973, the following companies supplied a total of 37,679 vials and 3,123 bottles of tablets and/or capsules through this joint research effort:

Bristol Laboratories, USA	NSC-26980	Mitomycin
Bristol Laboratories, USA	NSC-157365	Neocarzinostatin
Institut Merjuex, FRANCE	NSC-197213	C. Parvum
Ives Laboratories, USA	NSC-82151	Daunomycin
Mead Johnson Laboratories, USA	NSC-109724	Ifosfamide
Microbiological Research, ENGLAND	NSC-106977	Erwinia Asparaginase
Sandoz Pharmaceuticals, SWITZERLAND	NSC-141540	VP 16
Sandoz Pharmaceuticals, SWITZERLAND	NSC-122819	VM 26
E. R. Squibb and Sons, USA	NSC-23759	Testololactone
Spofa, CZECHOSLOVAKIA	NSC-104801	Cytembena
USSR	NSC-167781	Diiodobenzotepa
USSR	NSC-167780	Asaley
USSR	NSC-73754	Ftordopan
USSR	NSC-180024	Carminomycin
USSR	NSC-148958	Ftorafur
Winthrop Products, Inc., USA	NSC-142982	Hycanthone
Lederle Laboratories, USA	NSC-3590	Calcium Leucovorin
Upjohn Company, USA	NSC-70735	Nafoxidine

Purchase of Dosage Forms

A major activity of the Section involves the purchase of formulated products. These items fall into two categories: (1) investigational products and (2) commercial products. The investigational products are formulated proprietary drugs not available for commercial sale but of interest to the Cancer Treatment Program. Such products are usually manufactured according to NCI specifications and sold to NCI on a cost reimbursement basis. Commercial products are items available for open purchase.

Procurement of all purchased dosage forms is handled through the NIH Procurement Branch. Several procurement mechanisms are used depending on such factors as the type of product, the size of the order and the priority for clinical need. The most convenient and expedient form of procurement for high use items is a negotiated Indefinite Delivery Contract. During 1973 this Section initiated sixteen Indefinite Delivery Contracts.

A total of 777,724 vials and 22,303 bottles of tablets and/or capsules (2,120,300 oral doses) of commercial and investigational dosage forms were purchased during calendar year 1973. All suppliers are required to provide a certificate of analysis on each lot of drug purchased. The data are reviewed prior to release for clinical use.

Table XVII summarizes the procurement activities of the Section during calendar year 1973.

TABLE XVII
DRUG ITEMS PROCURED

SOURCE	Capsules/Tablets 1972	Capsules/Tablets 1973	Vials 1972	Vials 1973
Produced by Contractors	1,704,500	1,255,005	504,626	458,377
Contributed or Cost Sharing	7,500	208,710	138,357	37,679
Purchased Invest. Drugs	152,400	85,000	99,469	529,778
Purchased Commercial Drugs	2,666,600	2,035,300	200,752	247,946
	4,531,000	3,584,015	943,204	1,273,780

Quality Control of Clinical Dosage Forms

Each lot of formulated drug must meet specific quality control criteria prior to release for clinical use. This Section, in collaboration with the Chemical and Drug Procurement Section, maintains a strict quality control program designed to insure that each batch of formulated material meets the criteria for strength, quality and purity. Parenteral products are tested for potency, sterility, safety, pyrogenicity, etc., and oral products are tested for content uniformity, weight variation, hardness, disintegration, etc. All products are tested for potency by the formulator and sometimes by another laboratory.

Individual unit assays are required; the number of samples assayed depend on the dosage form and the size of the production batch. All new dosage forms are routinely submitted to an outside analytical contractor to verify the analytical methodology and accuracy of the formulator. The confirmation analyses are mainly performed by the contract analytical laboratories, Stanford Research Institute and Midwest Research Institute.

This Section participates in the activities of the Quality Control Committee for Chemicals and Drugs. This committee reviews for clearance the analytical results of bulk chemicals and formulated clinical dosage forms. During calendar year 1973, 252 lots of clinical dosage forms were reviewed by the Quality Control Committee for Chemicals and Drugs. Of these, 165 were investigational dosage forms and 87 were commercial dosage forms.

The pharmaceutical contractors are requested to perform compatibility and stability studies on reconstituted and diluted solutions of the investigational dosage form. These studies are carried out under simulated clinical conditions to test the effects of temperature, light and salt additives. The accumulated information is then reported on a Pharmaceutical Data Sheet which is incorporated into the Clinical Drug Brochure.

This Section also maintains a shelf-life surveillance program on all lots of non-commercial formulated dosage forms. Representative samples of each formulated lot are stored under specified controlled conditions and assayed for chemical and/or physical change. Three assay schedules are used for shelf-life studies: Schedule A, designed to provide stability data under accelerated conditions; Schedule B, designed for controlled studies of repeat formulations; Schedule C is assigned to products that have been prepared more than three times. The Schedule C samples are held but not assayed unless specifically requested. All Schedules provide a minimum two-year surveillance record.

Preparation of Pharmaceutical Data for FDA-IND Submissions

This Section prepares attachments I, II, III, V and VII for submission to Cancer Therapy Evaluation Branch for IND filing with the Food and Drug Administration. These attachments consist of the description, composition, method of manufacture and controls used in the preparation of the dosage form. The FDA requires that such a submission be made for each new investigational drug prior to clinical release. In addition, IND amendments must be submitted whenever a new dosage form of an existing IND drug is formulated for clinical use. During 1973 this Section prepared fifteen original IND reports and ten amended IND reports.

Storage and Distribution of Clinical Dosage Forms

The receiving and shipping activities of the Section are carried out by three shipping clerks. These clerks have been instructed in the required handling techniques for each drug. They are knowledgeable of postal and freight procedures used in this country and throughout the world. A significant amount of clinical drugs are shipped to foreign investigators each year. During 1973, approximately 946 foreign shipments were made. During the same period 10,061 domestic drug shipments were made.

During 1973 a total of 1,339,344 drug items (vials and bottles) were received and inventoried for subsequent worldwide distribution. During that same period, 1,131,801 drug items were distributed (100% increase over 1972 which had increased 30% over 1971) averaging 44 individual shipments daily.

This Section is also responsible for coding of drugs for double-blind studies. These studies may involve two or more drugs; the same drug in different dosage strengths, or a combination thereof. In all instances, these dosage forms are indistinguishable in appearance for double-blind purposes. The codes are placed on each bottle or vial by a supply clerk and checked by the pharmacist prior to shipment. There are presently thirteen such coded studies on inventory.

Another phase of the Clinical Drug Distribution program involves the management of returned drugs from clinical investigators. Returned drug information is recorded on the original inventory file, including the name of the investigator, the amount of drug returned and the date received. This information completes the distribution cycle of the drug since the original shipping date is also on file by investigator's name, quantity shipped, etc. This Section is responsible for the disposition of the returned drug. The judgment to restock, destroy or quarantine a returned drug is dependent upon the condition of the drug container, the age and known stability of the dosage form, the amount of drug in question and other factors.

During 1973 the Section handled an average inventory of 442 dosage forms by lot number involving 214 different formulations of 129 different compounds. These figures include investigational and commercial dosage forms.

Serial No.: 3584
1. Division of Cancer Treatment, DR&D
2. Drug Development Branch
3. Clinical Drug Distribution Section
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Research in Dosage Form Development of New Antitumor Drugs

Previous Serial Number: 3584

Principal Investigator: J. Paul Davignon

Other Investigators: James C. Cradock, John S. Driscoll and Harry B. Wood, Jr.

Man Years:

Total : 0.50

Professional : 0.50

General Objectives:

(1) Study new formulation approaches with potential utility for those compounds posing difficult formulation problems.

(2) Evaluate dosage form problems presented by clinical and laboratory investigators.

Specific Objectives:

(1) Development of a parenteral dosage form for NSC-95441 (Methyl CCNU) and NSC-79037 (CCNU).

(2) Evaluation of the effects of a non-ionic surfactant on solubility of certain poorly water soluble antitumor agents.

(3) Formulation of a more suitable parenteral dosage form of NSC-71795 (Ellipticine).

(4) Development and formulation of a stable preparation of NSC-153858 (Maytansine).

Major Findings

(1) Development of Parenteral Forms of NSC-79037 (CCNU) and NSC-95441 (Methyl CCNU).

CCNU and Methyl CCNU are insufficiently soluble in water or dilute (5-10%) ethanol solutions. Solubility problems were obviated by dissolutin of the compounds in an equal volume mixture of ethanol and polyethoxylated castor oil (PECO), a non-ionic surfactant, and further dilution with intravenous fluids. A clear yellow solution is obtained and this solution is administered by slow (1-2 hour) infusion. The formulation is supplied as a two component package consisting of sterile dry nitrosourea and an ampoule of the nonaqueous diluent. The preparation is reconstituted and diluted aseptically immediately before use. Protocol toxicology has been completed by the Laboratory of Toxicology on vehicle alone as well as on both nitrosoureas in this solvent system and clinical trials have commenced.

Although PECO resolved a solubility problem, vehicle interference with UV spectra of the nitrosoureas necessitated estimtation of stability by pyrolysis gas chromatography and colorimetric methods. An extraction procedure recently developed by the Drug Design and Chemistry Section, DDB, provides separation of nitrosoureas and PECO and direct assessment of drug stability by absorption. Both the CCNU and Methyl CCNU formulations are stable beyond the intended period of us (> 2 hours).

(2) Solubilization of Certain Antitumor Agents Using a Non-Ionic Surfactant

The applicability of PECO in the formulation of compounds exhibiting excellent solubility in ethanol, dimethylacetamide or dimethyl sulfoxide but minimal water solubility was evaluated. Vehicle systems containing equal parts of nonaqueous solvent and surfactant and yielding a clear solution after dilution with ∿ 10 volumes of intravenous fluid appear promising. The antitumor effects and toxicity of several such solubilized preparations including NSC-403169, NSC-141549 and NSC-143414 have been evaluated in rodents by the Drug Evaluation Branch. This solubilization approach may have utility with a variety of pharmacologically active agents.

Recently several reports appeared concerning intravenous administration of parenteral suspensions and emulsions of delta-9 tetrahydrocannabinol (THC), an active principle of marijuana. Since THC is ethanol soluble and a solution is preferred for I.V. use, the effects of PECO were evaluated. Use of a simple dilution and mixing procedure yielded clear, tan colored, solutions of THC 0.5-10 mg/ml in Ethanol-PECO-Normal Saline 5:5; 90. With the cooperation of the Laboratory of Toxicology, the effects of THC following I.V. administration were studied in the rabbit. Twitches, convulsions, vasodilation and several deaths were noted following 100 mg/kg. Hyperactivity but no lethality was observed at 40 mg/kg. Equivalent amounts of vehicle (∿7 ml/kg) produced only slight ataxia and depression.

(3) Formulation of NSC-71795 (Ellipticine)

Ellipticine-induced hemolysis and hypotension as well as low solubility present problems in parenteral dosage form design. Slow infusion of dilute solutions (0.5-1 mg/ml) containing citrate buffer appears to have resolved these problems. Toxicology studies are in progress.

(4) Formulation of NSC-153858 (Maytansine)

NSC-153858 exhibits antitumor activity in experimental tumor systems at very low doses. Although maytansine has a low solubility in water, a suitable formulation was obtained by lyophilization of dilute ethanol solutions containing maytansine-mannitol 1:120. The formulation meets chemical and biological and toxicology studies will be initiated in the near future.

Miscellaneous Projects

(1) NSC-145668 (Cyclocytidine)

Reconstitution of lyophilized formulations of this compound yielded hazy solutions. The degree of opacity was related to heat, length of lyophilization cycle and rubber closure. Freeze drying for shorter periods in absence of closures resulted in a product of acceptable clarity.

(2) NSC-141540 (VP-16-213)

VP-16-213 is a podophyllin derivative supplied to the program in a non-aqueous multicomponent vehicle for subsequent dilution with intravenous fluids and administration by slow infusion. After receipt of reports concerning precipitation during infusion, the physical stability of VP-16-213 was evaluated in infusion solutions. The formulation is considerably more stable in Sodium Chloride Injection, U.S.P., than in 5% Dextrose solution. Dilution with twelve to twenty parts of normal saline solution yields a preparation of acceptable clarity for six hours.

About eight other small projects were carried out in the formulation laboratory in cooperation with other groups in the Division of Cancer Treatment. These included lyophilized and liquid fill preparations that were subsequently evaluated for pharmacological and/or toxicological effects.

Publications

Cradock, J. C., Davignon, J. P., Litterst, C. L., and Guarino, A. M.: An Intravenous Formulation of delta-9-tetrahydrocannabinol Using a Non-ionic Surfactant. J. Pharm. Pharmac.: 25:345, 1973.

Davignon, J. P., Yang, K. W., Wood, H. B., and Cradock, J. C.: Formulation of Three Nitrosoureas for Intravenous Use. Cancer Chemother. Rep. Part 3, 4: 7-11, 1973.

CHEMICAL AND DRUG RECORDS SECTION

DRUG DEVELOPMENT BRANCH

SCOPE

The responsibilities of the Chemical and Drug Records Section are (1) to acquire chemical compounds for the primary screen and for other program needs, (2) to develop, maintain and update systems of registry for all chemicals entering the program, (3) to maintain adequate storage and inventory of bulk chemicals and drugs, and (4) to coordinate the distribution of materials. These objectives are achieved through the efforts of staff members and the compound acquisition contractor to cultivate the interest of and to provide meaningful liaison with suppliers and potential suppliers; the surveillance of world-wide literature for new sources of chemical and drugs; the development of chemical nomenclature for all files; the distribution and utilization of chemical data; and the assurance that all discreet compounds obtained from commercial sources are maintained in accordance with the signed agreements.

STAFF

During this report period the Section was staffed with three chemists, one biologist, one secretary and two part-time clerk typists.

ACQUISITION OF COMPOUNDS

Materials entering the screening program for the most part are voluntary contributions from research investigators at universities, government agencies, various non-profit institutions, industries and individuals interested in the area of chemotherapy. This continuous voluntary input of samples results from correspondence and personal visits of staff members to these various institutions, and the collection efforts of the compound acquisition contractor, Starks C.P. Inc. This Contractor, through diligent liaison efforts, was able to acquire 30,154 samples of which approximately 18% were duplicates. The majority of compounds collected (21,213) were from industry. Of the remaining samples, 5,206 were collected from government laboratories, 3,735 were from academic research laboratories, and 878 were purchased.

Effective liaison work by NCI Liaison Officers in Europe and the Far East are playing important roles in the acquisition of samples for the primary screen. The input of most new samples from these efforts did not begin to take place, however, until the last quarter of this reporting period. Six hundred seventeen (617) compounds were assigned for testing in Italy, 290 compounds in Belgium, and 174 compounds in Japan. In addition five compounds were received for testing from the U.S.S.R.

During calendar year 1973 the major portion of the liaison efforts were performed by the compound acquisition contractor and by NCI Liaison Officers in Europe and Japan. A breakdown of the Starks C.P. Inc., activities show that 84 industrial sources, 99 academic sources and 5 government laboratories were contacted. In addition, Starks C.P. Inc., on a limited scale, surveyed the literature for compounds that were designed specifically for non-cancer related biologic testing. Ninety-nine (99) individual researchers were contacted and 205 compounds were acquired in this manner.

During this reporting period 262 new suppliers were added, of which 162 were new non-industrial suppliers. Among the active compounds 93 new ring systems have been identified, bringing the total to 384 active ring systems. The Section has checked the publication status of the compounds reported in 130 manuscripts submitted for publication clearance.

The following chart gives a breakdown by sources for the new compounds registered in 1973:

COMPOUNDS SOURCES	NUMBER	PERCENT
Non Industrial	12379	36.0
Industrial	19845	57.6
Purchased	878	2.5
Contracts	1332	3.9
	34,434	100.0

To obtain 34,434 compounds it was necessary to process approximately 41,000 samples with about 18% duplicates.

Chemical Information

The Section cooperated with the Data Processing and Computer Systems Development Section in the registering of the total chemical file for computerized information retrieval. All new submissions are registered and duplicate checks are made automatically. Significant support has also been provided by Staff members in the development of the new chemical information system under contractual arrangement with the University of Pennsylvania. For rapid retrieval of chemical information Master Chemistry Card Files and Molecular formula files are maintained.

Structure-Activity Studies

A symposium on the current "state of the art" for structure activity was held on March 4, 1974. The results from this symposium and additional information concerning this area of activity are covered in the DDB Branch Report.

Other Related Duties

In compliance with the order issued by the Food and Drug Administration, the Section monitors and maintains records of input and distribution of materials considered by FDA as narcotics, barbiturates, hallucinogenic and other dangerous drugs. The FDA has specified that separate and accurate records be maintained for these materials and only these individuals with the proper narcotic license be approved to dispense and receive these items. To insure proper safety, the drug abuse compounds are secured in a separate file under lock and key.

The confidential status of compounds obtained from industrial sources requires constant up-dating and careful monitoring. The recognition by these organizations that the Branch maintains constant surveillance over commercial discreet materials has afforded not only a good industrial relationship but has made available many commercial samples not otherwise accessible. Frequently, there is a need to obtain the release of compounds from confidential status. Declassification is achieved only when the industrial supplier agrees, in writing.

Chemical and Drug Distribution

This Section is also responsible for the receiving, storage and distribution of chemicals for screening. The Section is charged with the custody of bulk chemicals and drugs that enter the Program. Detailed inventory records, such as source, date received, amount, lot number, initiator of all shipments, and destination of drugs are recorded on a daily basis.

The Section monitors the portion of the contract with Microbiological Associates, Inc., (N01-CM-33728) which handles the receiving, weighing, recordkeeping, distribution and storage of compounds. Over 200,000 different materials are held in storage. These consist largely of chemicals and to a lesser extent plant products, antibiotic and steriods. A total of 25,530 new compounds were shipped to the screening laboratories; 32,740 refills and materials for special testing were weighed and 3,676 packages were shipped. During 1973 approximately 8,436 samples were returned by the screening laboratories. These compounds, for which testing has been completed, are entered into the inventory.

Areas Under Development

The Section is in the process of assembling meaningful information to aid in the development of guidelines for discriminate selection of compounds. Informal discussion with experts involved with the acquisition of compounds for biologic testing have not provided definitive guidelines that could be easily applied to the NCI program.

Contracts

During the past year the Section has participated in the monitoring of the contracts listed in Table XVIII.

TABLE XVIII

CONTRACTS MONITORED BY THE SECTION

CONTRACTOR	INVESTIGATOR	CONTRACT NUMBER
Chester Beatty Research Institute	Connors	N01-CM-43736
Microbiological Associates, Inc.	Berman	N01-CM-33728
Starks C.P. Inc.	Starks	N01-CM-23705

DATA PROCESSING AND COMPUTER SYSTEMS DEVELOPMENT SECTION

DRUG DEVELOPMENT BRANCH

Scope

The Section is primarily concerned with: (1) directing a major effort to implement a new automated Chemical Information System (CIS) which supports such activities as the accessioning, storage, and distribution of compounds acquired for antitumor testing; (2) operating the CIS system; (3) coordinating many of the activities related to the CIS. In addition, the Section cooperates in projects to explore the usefulness of automated literature and chemical files maintained by Federal agencies, industrial organizations, and information vendors.

Staff

The staffing of the Section consists of two system analysts and one secretary.

As part of the reorganization of the Drug Research and Development activity, the Data Processing and Computer Systems Development Section was transferred to the Drug Development Branch (DDB) with the responsibility to develop data processing systems to meet the scientific, operational control, and management planning needs of DDB.

Emphasis of Effort

During 1973 the Data Processing and Computer Systems Development Section concentrated on implementing the first phase of the new Chemical Information System, procuring and orienting the contractor to operate the new system, managing the orderly transition from the previous semiautomated system to the new system, and coordinating the interaction with the system by the DDB staff and the acquisition, system operation, and storage and distribution contractors The use of computer tape services was continued to identify newly synthesized analogs of compounds of interest which were reported in the literature and action initiated to acquire such compounds from foreign and domestic suppliers.

The Section also cooperated in the effort to assess the value and effectiveness of Cancer Chemotherapy Reports and Cancer Therapy Abstracts (formerly called Cancer Chemotherapy Abstracts) in disseminating cancer therapy information to investigators both within and outside the Division of Cancer Treatment Program. Technical support was provided in converting the Cancer Therapy Abstracts data available in a machine-processable form to a file formatted for inclusion in the International Cancer Research Data Bank.

Implementation and Installation of the Phase I Chemical Information System

The University of Pennsylvania has implemented and installed the first phase of the Chemical Information System (CIS) at the Chemical Abstracts Service (CAS), the operation contractor for the new system. This phase integrates the newly-developed CAS Registry III programs into a single, comprehensive system to handle a wide range of sample processing activities, ranging from structure input to the printing of reports of NSC assignment and reports for management.

The information retrieval component of the system was completed during 1973 and is scheduled to be installed in July, 1974. Using a graphics terminal with simplified, computer-supported techniques, the system will permit access to the files for full structure search (to secure an immediate answer for a possible duplicate sample) and substructure search (for analogs). The new system will provide more rapid duplicate checking than now possible, will begin to give structures as output, and will allow extremely fast retrieval from the chemical file as compared to present methods.

Implementation of the Phase II Chemical Information System

The University of Pennsylvania began the implementation of the second phase of the CIS which is scheduled to be operational in March, 1975. This phase will extend the system to automate shipping procedures and maintain inventory control. Submissions that are duplicates or are closely related to known active compounds will be automatically brought to the attention of management or automatically assigned to specialized testing procedures by programmed decisions. The retrieval capability will be considerably enhanced.

Operation of the Phase I Chemical Information System

In April, 1974, the Chemical Abstracts Service (CAS) was chosen as the operator of the new CIS. CAS has operated the current chemical information processing system. During the final quarter of preceding contract(N01-CM-51021) and the first quarter of the new contract (N01-CM-43722), CAS provided developmental products and support services for the implementation and installation at CAS of the Phase I CIS. To assure the optimal effectiveness of the new system, this Section has maintained continuous, coordinated liaison with the contractors involved in the system as well as with the DR&D staff.

Literature Searches for Selective Compound Acquisition

Computerized searches for analogs of interest to this program from among newly synthesized compounds reported in the literature were continued with the cooperation of the Scientific Records Section (SRS). A successful preliminary search of the Index Chemicus file maintained by the Institute for Scientific Information was made for the ANSA class of antibiotics, including Rifamycins, Geldanomycins, Streptovaricins, Tolypomycins, and the Maytansines. Consequently a five-year retrospective search has been initiated.

Action was initiated with the acquisition contractor to acquire 72 analogs of compounds of interest reported in the literature and 220 analogs identified by a computerized search of the Shell Development Co. file. Similar action was initiated with the European Liason Office to acquire 152 analogs from existing and potentially new foreign suppliers.

Cancer Chemotherapy Reports and Cancer Chemotherapy Abstracts Evaluation Study

An evaluation study of *Cancer Chemotherapy Reports* and *Cancer Chemotherapy Abstracts* is in progress to assess the value and effectiveness of these publications in disseminating cancer therapy information to investigators both withi and outside the Division of Cancer Treatment Program. This study was made as part of the International Cancer Research Data Bank Services Study. The project encompassed selection of the audience and the survey mechanisms and the development of questionnaires for domestic and foreign individual and institutional subscribers. User perception of the publications covered such characteristics as timeliness, editorial quality, coverage and the relationship of the publications to users needs.

Cancer Chemotherapy Abstracts On-Line Data Base

Technical assistance was provided to Informatics, Inc., the contractor responsible for carrying out the International Cancer Research Data Bank (ICRD Survey Study, in building the first on-line literature file in the ICRDB. Some 15,000 abstracts published from 1967 through 1971 in *Cancer Chemotherapy Abstracts* (*CCA*), available in a machine processable form (MTST tapes), are being reformatted and converted to a computer tape. Computer tapes containing abstracts and index terms had already been generated for abstracts published since 1972 as a by-product of preparing camera-ready copy of *Cancer Chemothera Abstracts*. Since 1972 approximately 3,000 abstracts have appeared yearly. The resulting file of *CCA* abstracts with index terms is planned as one of many data bases to be searched by the on-line system (TOXLINE) of the National Library of Medicine as one module of the ICRDB.

Miscellaneous

The Section coordinated the collection of information required by NIH, DHEW, and GSA for the Automated Data Processing and Management Information Reporting System.

In the coming year, the Section will concentrate on administering and managing the contracts with the Chemical Abstracts Service (CAS) and the University of Pennsylvania. The CAS contract will include the operation of the Phase I CIS and support in the installation and operation of the Phase II System. The University of Pennsylvania contract will provide for the implementation and installation of the Phase II CIS and the participation in evaluating the effectiveness of the new system.

TABLE XIX

CONTRACTS MONITORED BY THE SECTION

CONTRACTOR	PRINCIPAL INVESTIGATOR	CONTRACT NO.
University of Pennsylvania	Lefkovitz	NO1-CM-33719
Chemical Abstracts Service	Seals	NO1-CM-43722

SCIENTIFIC RECORDS SECTION
DRUG DEVELOPMENT BRANCH

Scope

The Scientific Records Section (SRS) stores, analyzes, and dispenses the majority of the chemical information used in the development of the Division of Cancer Treatment's drug research program. In order to fully utilize the files of chemical and biologic data that have been built in the past 18 years, the Section has the ability to search the chemical file by structure or substructure and to provide data such as structural formula, systematic name, and biologic data in various formats to requesters. A major portion of the data for structure-activity studies are gathered by this Section with the cooperation of other parts of the Drug Development Branch (DDB) and Drug Evaluation Branch (DEB) and new methods in data handling are continually investigated to facilitate such correlation.

The Section prepares and maintains the master structure file and several cross-reference files on each compound. It coordinates the organization of new compounds into files suitable for retrieval. The Section also prepares shipping lists for the transmittal of compounds to screeners using the various special shipping instructions for certain categories of agents.

Staff

The staff of the Scientific Records Section consists of 3 chemists, one chemical information clerk, 3 clerk typists and part time clerical help including either a stay-in school employee or clerk typist trainee. During this report period the Section and the majority of its former activities were transferred to the Drug Development Branch. The Section personnel responsible for monitoring the biologic data processing system were transferred to the Drug Evaluation Branch.

Chemical File

The structures of all substances acquired by the Drug Development Branch (DDB) are transferred to a standard card according to Chemical Abstract structuring conventions. The cards are used as input to a computer registry system maintained under contract by Chemical Abstracts Service (CAS)(NO1-CM-51021). The system checks for duplicates to allow the assignment of an NSC number and systematic chemical names are generated for new compounds.

The Section structured new input and assigned the majority of the systematic nomenclature for the new compounds entering the program. The number of compounds assigned NSC numbers went from 15,770 in calendar year 1972 to 34,434 in calendar 1973, with actual raw input rate before duplicate checking reaching 41,000 per year.

Shipping

The Section prepares material shipping lists (MSL) for the new compounds prior to their shipment to the screening laboratory. The MSL's provide the initial input to the biologic data processing system. Shipping lists for 28,290 compounds were prepared during calendar year 1973.

Name and Formula Indexes

During the past year a large number of USAN synonyms were added to the file by checking the master USAN list containing CAS registry numbers, against the CAS registry number to NSC cross-reference list prepared from the bibliography file. The USAN synonyms are official synonyms adopted by the USAN Council and are thus disseminated nationally. An effort is being made to emphasize these synonyms at DCT in preference to trivial names that are often used. Additional CAS registry numbers were added to the file during the year from a computer comparison of the NCI and CAS registry files. There are now approximately 90,000 CAS registry numbers listed as synonyms.

Microfilming

The chemistry card file was filmed on a continuous basis during the year and the film is current through NSC 197500. The large influx of compounds and slow turnaround at the Federal Records Center have resulted in a backlog in the filming of chemistry cards. It is anticipated that this situation will be eliminated during the next year. The name and formula indexes were filmed in 1973 using a special printout-to-film camera. SRS also collaborated with the Automated Information Section, DEB, to produce COM microfiche of a supplier-NSC index that was previously stored as bulky printouts.

Chemical File Analysis

In addition to maintaining access to the chemical data bank, the Section provides advice on its use, produces special reports, and investigates new methods to organize and handle this data.

An investigation into the use of pattern recognition techniques was undertaken in cooperation with scientists at the Division of Computer Research and Technology (DCRT). The computer connection table for compounds tested against 3EM37 were broken by computer into 'fragments'. These fragments were then used as 'attributes' and a model was built to analyze additional structures. The initial results were promising and an investigational base for further computer analyses of the file was established. The Section is cooperating with other members of DDB staff in the practical uses of pattern recognition in the drug development effort.

Substructure Search

Through computer programs developed at CAS, the Section has the ability to search all chemicals in the file for selected fragments. Eighty-eight full searches of the file were run during the year, together with 48 updates to searches previously run. These included 26 active analog searches which were run primarily for the purpose of aiding the Drug Evaluation Committee and Decision Network meetings in evaluating the biologic data of a compound in light of its structural novelty.

Developments in Search

The Section cooperated with the University of Pennsylvania in the development of the structure handling portions of the new chemical information system. Substructure search questions were phrased for the CAS search system in order to retrieve peculiar structure types that were used to test the search system fragment assignment programs. Consultation on the actual keys used and some system checkout of programs were also provided. Section personnel reviewed the new methods available in this system that will allow faster, cheaper searches in the coming year.

Special Projects

As part of the file-review process for the new chemical information system, the Section was responsible for two major updates of current files. Discrepancies in the supplier code, discreet code and publication code between the chemistry file and the biologic file were reviewed and the necessary corrections were provided to update the file. In addition, the master name and address file was reviewed and updated to convert it to the more comprehensive data needed for the new system. The file was reviewed supplier by supplier to put in such data as geographic code, mailing codes and report-to codes. In addition, the names and addresses of deceased suppliers were reinserted to give an historical record of how every compound was submitted.

CONTRACT MONITORED BY THE
SCIENTIFIC RECORDS SECTION

TABLE XX

Contractor	Investigator	Contract Number
Chemical Abstracts Services	Brown	NO1-CM-51021

Publications By Contractors

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methyl]oxiranes. *J. Pharm. Sci*., 62: 1894, 1973.

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ANNUAL REPORT

DRUG EVALUATION BRANCH

DRUG RESEARCH AND DEVELOPMENT

DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

The objectives of the Drug Evaluation Branch (DEB) are to initiate, develop, conduct, and coordinate experimental laboratory projects for the recognition, recommendation, and preclinical development of new and improved drug therapies for use in the treatment of human cancer. Objectives are achieved by (a) screening large numbers of synthetic and natural products for biological activities indicative of potential clinical usefulness, (b) evaluating "active" drugs with respect to conditions of usage, including treatment route and schedule, combination chemotherapy, and combined treated modalities, which may enhance therapeutic efficacy, (c) studying means of altering tumor cell sensitivity and treatment by altering antigenicity, (d) investigating biochemical and cellular sites of drug action, (e) investigating the physiological disposition and metabolism of active drugs with respect to their influence on therapeutic efficacy, (f) studies of the role of drug interactions on the effectiveness of combination treatment, (g) investigating related specific problems arising during preclinical toxicological examination, clinical formulation development, and clinical trial, and (h) developing new and improved methods for preclinical drug evaluation. Studies are aimed at providing information essential to achieving objectives set forth in the DCT linear array, at research contributing directly to the development of methods for providing such information, and basic studies of significance to understanding scientific principles that could have a major impact on program objectives. Program is implemented through contracts with highly qualified institutions, through intramural laboratory projects conducted within DEB, and through collaboration with other NCI projects.

Major accomplishments during the year included completion of studies of the correlation between drug activity in animal screens and clinical activity. The study showed that the predictive value of mouse leukemias L1210 and P388 extended to the treatment of human solid tumors. The value of the relatively resistant B16 mouse melanoma and Lewis lung mouse carcinoma (LL) resided in their ability to select from drugs that pass the initial L1210 and P388 screens those with the best chance of efficacy against major classes of human solid tumors. Accordingly, the drug screening procedure was modified so that new synthetic agents and natural products are screened initially against L1210 and P388. Those that emerge as active are screened in the more costly B16 and LL systems. The study also showed that priorities for the further development of drugs to the clinic should be based on the qualitative and quantitative aspects of their activity against the four animal tumors selected.

The "mini-screen" concept was implemented for the initial screening of "random" new synthetics and crude natural products in order to accommodate a substantial increase in the input into the screen from approximately 30,000 materials in calendar year 1972 to approximately 50,000 in calendar year 1973 and to provide resources for the secondary screening (B16 and LL) and more detailed evaluation of active drugs. Consequently, despite the 67% increase in input into the screen, the cost of biological testing related to bioassay of natural products (DCT Array Code - IXX) increased by only 9%, and the cost of testing related to screening of new materials (2AX) increased by only 6%. However the cost of testing related to the detailed evaluation of active drugs and to combination therapy (2B2) increased by 32%. Overall, the total cost of DEB monitored contract projects increased by 10%.

A substantially increased emphasis in the study of combined therapies, both drug oriented and disease oriented, accounted for most of the increased effort in areas of detailed drug evaluation and these studies are described in more detail later in this report. Proceedings of Screeners' Workshop on Combination Therapy were published in Cancer Chemotherapy Reports, Part 2, Vol. 4, March 1974 and cover recent advances within our program with respect to studies of combinations of drugs, drugs plus surgery, drugs plus immunotherapy, and drugs plus radiation. Emphasis was placed on the future of combined modalities of treatment, rationales for the selection of drug combinations for preclinical evaluation, and the complexities of experimental design and analysis of results inherent to combined therapy studies.

During the past year, reorganizations within DR&D have had a major impact on the structure and functions of the Branch. The Molecular Biology and Methods Development Section (MBMD), was established in August, 1973. The objective of MBMD is to monitor contract studies and conduct intramural research leading to the establishment of new and improved biological and biochemical drug evaluation assays. The MBMD Section will coordinate and review program sponsored projects related to (a) the growth kinetics of tumor cells and normal cells *in vivo* and *in vitro*, (b) the relative effects of antitumor drugs on the growth of tumor cells and critical normal cells as well as on biochemical sites of inhibition, (c) the development of novel *in vitro* and *in vivo* screens of greater predictive value for specific organ directed anti-tumor specificity and (d) the development of rational bases for the prediction of treatment schedule dependency and optimally effective drug combinations.

During the past year, the intramural laboratory efforts conducted by the Screening Section, DEB were transferred to the new Tumor Biology Section (R. Johnson, Head) established within the Laboratory of Experimental Chemotherapy (A. Goldin, Chief). The Program Analysis Branch, DR&D, was dissolved and biological data processing functions formerly managed by PRA were assigned to DEB. The Automated Information Section (AIS), DEB, was established to carry out these functions.

Currently, DEB includes the Office of the Chief (J. M. Venditti), and four sections: Automated Information (M. M. Macdonald, Acting Head), Biochemistry (F.R. White, Head), Molecular Biology and Methods Development (V.H. Bono, Head), and Screening (B.J. Abbott, Head) and is staffed by 12 senior professionals (including 3 commissioned officers and 1 staff fellow), and 19 junior professional, technical, and clerical personnel. Seven of the latter 19 are parttime or employed under "Stride" or "work-study" programs.

DEB is responsible for the direct management of 25 contracts and one PL-480 agreement and for drug evaluation activities under nine contracts and two PL-480 agreements administered by other DCT units. Obligated or estimated funds for DEB monitored activities as of February 28, 1974 totaled approximately $10,364,000/a. Funding of DEB monitored contract activities with respect to DCT linear, supplementary, and exploratory arrays, and description of the tasks carried out by each Contractor are listed in Tables 1 and 2. In addition, DEB intramural laboratories conducted projects related to basic studies for drug evaluation utilizing antitumor screening (see current Indivudual Project Report, Serial No. NCI-3578), basic studies on the modification of division patterns and immunogenicity of neoplastic cells by interaction of their surfaces with microenvironmental molecules (see current Individual Project Report, Serial No. NCI-3583), and basic studies of the effects of drugs on the cycle progression of L1210 cells _in vitro_ (see current Individual Project Report , Serial No. NCI-6103).

During calendar year 1973, 51,334 synthetic and natural products were submitted to DR&D for screening and 50,984 materials were screened. This represented an approximately 67% increase in input to the screen from the previous year. Approximately 67% of the materials submitted in 1973 were synthetic agents. A total of 395,152 screening tests were conducted; 356,115 _in vivo_ antitumor tests, 31,820 cell culture tests, and 787 miscellaneous tests. Of the _in vivo_ antitumor tests, 134,562 were conducted against mouse leukemia L1210, 198,723 against mouse leukemia P388, 15,989 against mouse B16 melanoma, 2,441 against mouse Lewis lung carcinoma (LL), 2,049 against mouse AKR lymphoma, and 2,351 against other tumors. Ninety compounds were reviewed by the Drug Evaluation Committee and ten passed DCT-Decision Network (DN)-2A. The latter are listed in the current organizational report of the Screening Section, DEB.

Continuing studies of correlations between clinical efficacy and animal antitumor activity confirmed the previous finding that L1210 predicted most of the known clinically useful drugs and should be continued as our initial primary screen for new synthetic materials. In our last annual report we described studies suggesting the use of the "mini-screen" approach in the screening of new synthetics. Also, we described studies showing that P388 was more sensitive than L1210 to crude natural products leading to the decision that these materials be screened initially against P388. Although B16 and LL were known to be generally less responsive to chemotherapy than L1210 or P388, the potential value of the slower-growing B16 and LL tumors as secondary screens was indicated by their unusual sensitivity to certain clinically effective drugs that were not predicted by L1210 and P388.

Improvements in the therapy of human cancer have been achieved largely against relatively rapidly growing leukemias and lymphomas. Currently, the drug screening aspect of the DCT program is aimed at uncovering drugs with a high likelihood of activity against human solid tumors. Within the past year, DEB concluded a comprehensive study of correlations between activity against L1210, P388, B16, and LL and activity against human solid tumors. Forty-five drugs regarded as adequately evaluated against at least two human solid tumors were analyzed. The drugs were classified as follows: 12 drugs with definite activity against at least two major solid tumor types in man, nine drugs which are clearly active against solid tumors in man but less active than the highly active drugs, 12 drugs for which some activity against solid tumors in man has been reported but not clearly established including those active against one solid tumor type but clearly inactive against a wide range of others, and 12 drugs which have been clearly inactive against at least two human solid tumors.

At the 25% increase in lifespan (ILS) cut-off for activity, L1210 selected 14 of 21 human solid tumor active drugs, but also 13 of 24 marginally active and inactive drugs. Thus, L1210 had the ability to uncover a high percentage of true positives, but also selected a high percentage of false positives for human solid tumor activity. The discriminatory value of L1210 increased as the criteria for "activity" increased. Thus, only 52% of drugs in this study eliciting an ILS ≧50% against L1210 were active against human solid tumors. However, 64% of those producing an ILS ≧100% were active against human solid tumors and 84% of those producing an ILS ≧200% showed human solid tumor activity.

P388 responded very much like L1210 in that it would be expected to miss very few drugs known to be active against human solid tumors. Also, the probability of P388 selecting false positives for the clinic is high when the criteria for P388 "activity" is low and decreases sharply as the criteria for "activity" is increased. Thus, 59%, 70%, and 100% of drugs eliciting ILS's of 50%, 100%, and 200%, respectively, against P388 were active against human solid tumors.

B16 does not appear to be a good choice for an initial screen because it missed nine of 21 clinically active drugs at the 50% ILS level. Also, it is 2 1/2 - 3 times more costly than the L1210 or P388 screens. None of the drugs in this survey were selected for clinical trial on the basis of B16 activity. Therefore, the observations that 100% of the drugs that achieved an ILS ≧100% against B16 were active against human solid tumors suggests B16 as an excellent ancillary model for selecting, from among drugs that pass the L1210 or P388 screens, those with the greatest likelihood of human solid tumor activity. B16 would be useful in establishing priorities for development to clinical trial among drugs that pass initial L1210 or P388 screens.

LL is very resistant to chemotherapy and relatively costly. It would not be suitable for a primary screen. However, the four drugs that produced an ILS ≧50% against LL were active against human solid tumors. The results indicate that LL, as B16, should be used as a secondary screen for establishing priorities for development to clinical trial.

The analysis described above showed that the predictive value of L1210 extends to human solid tumors as well as leukemias and lymphomas. Critical evaluation of a number of transplantable animal tumor systems has led to the selection of L1210 and P388 as the tools of greatest utility for the screening of synthetic agents and natural products, respectively. P388, in addition to its usefulness for the screening of crude natural products and for bioassay of natural product fractions, appears to be at least as predictive as L1210 for agents that are active against human solid tumors. Indeed P388 appears to be more sensitive than L1210 to the clinically most useful representatives of most major classes of antitumor drugs, although neither L1210 nor P388 are remarkably sensitive to hormonal agents or agents designed to enhance host immune responses. The question of whether P388 may eventually surpass L1210 as the primary screening tool in the DCT drug developmental program will requir more study. Currently, P388 is used for the initial screening of crude natural products. Those that elicit a reproducible ILS ≥25% against P388 are fractionated (using cell culture screens if appropriate), and the purified active component is then screened against both L1210 and P388. Purified natural products that meet minimal requirements to "pass" the P388 (ILS ≥50%) or L1210 (ILS ≥25%) screens are then tested against B16 and LL. Synthetic agents are screened initially against L1210 and those that elicit a reproducible ILS ≥25/ are then screened against P388, B16, and LL. Previous experience suggests that if 50,000 materials were entered into the initial L1210 or P388 screens, only a few hundred would meet activity criteria sufficient to prompt screening in the more costly B16 and LL.

The value of the B16 and LL screens appears to reside in their capacity for selecting a few drugs with the best chance of efficacy against human solid tumors from among those that pass the L1210 or P388 screens. The results of this study indicated that the likelihood of clinical activity increases with increasing degree of activity in any one of the four tumor systems (really five systems since both IP and SC inoculated B16 are used) and with the number of responding animal tumors. At present, drugs are recommended as candidates for development to clinical trial if important activity in one of the models is observed (L1210 - ILS ≥50%, P388 - ILS ≥75%, B16 - ILS ≥50%, or LL - ILS ≥50%). However, priorities for development to clinical trial among agents that meet one of these criteria are established on the basis of the qualitative and quantitative aspects of responses among all of the animal models.

Drugs that pass DN-2A of the DCT linear array are routinely tested for treatmen route and schedule dependency, that is, the degree to which variations in route and schedule may influence therapeutic efficacy. The protocols used for such testing, the patterns of response observed in the L1210 system and the relationship of in vivo results to the kinetics of tumor cell proliferation, biochemical sites of action, and drug pharmacology have been previously described. During calendar year 1973, 26 compounds were examined for route and schedule dependency and the results were reported to the appropriate DCT groups. Twenty-nine drugs were examined in the L1210 or P388 systems and eight were examined against B16 or LL. In general, the experimental protocols used for schedule dependency testing against the slower-growing animal tumors have been based on the protocols used for L1210 with some minor modifications relate to the duration of total treatment. We plan to examine the question of schedul dependency against B16 and LL in more detail and to design rationally based protocols for these tumors taking into account their growth characteristics.

Major emphasis related to the detailed evaluation of active drugs has been in the area of combination chemotherapy. In response to the needs of DCT Combination Chemotherapy Subcommittee (S. Carter, Chairman), DEB with the collabora tion of the Office of the Associate Director for DR&D (A. Goldin and R. Johnson has channelled the drug combination projects conducted at Microbiological Associates, Inc. (NO1-CM-33728), Southern Research Institute (NO1-CM-12098), and Arthur D. Little, Inc. (NO1-CM-33727) to the study of critical aspects of this problem. One basic question is the degree to which combinations of active drugs should be "screened" for potentiation and synergism. It is clear from our discussions with DCT clinical oncologists that the combinations in use or planned for clinical trial are selected mainly on the basis of established clinical experience with the individual drugs, e.g., therapeutic efficacy coupled with qualitatively different toxicities. The greatest clinical need at this time appears to be information regarding the best way to use such combinations. Combinations of other experimentally active antitumor drugs or combinations of an active drug with an inactive drug might be considered but only if the rationale for the synergistic observations (e.g., based on cellular or pharmacokinetic data, mechanisms of action, or resistance, drug metabolism or physiological distribution, etc.) is clearly defined in a number of systems so that carry-over to the human situation might be reasonably expected. Therefore, DEB efforts have been centered around detailed studies of combinations of immediate clinical interest. Both the drug oriented and disease oriented approaches have been pursued. With respect to the drug oriented approach emphasis was placed on combinations of Adriamycin (ADR, NSC-123127) plus other key drugs.

Studies using L1210 or P388 conducted at Microbiological Associates, Inc. and the Southern Research Institute, respectively showed that combinations of ADR plus Methotrexate (NSC-740) or ADR plus Melphalan (NSC-8806) were clearly synergistic against either leukemia. Against L1210, ADR plus Vincristine (NSC-67574) or ADR plus Me-CCNU (NSC-95441) were somewhat but not remarkably more effective than the most effective individual drug, but these combinations were clearly synergistic against P388. ADR plus 5-Fluorouracil (5-FU, NSC-19893) and ADR plus Anhydro Ara-C (NSC-145668) have not been synergistic against L1210 whereas the ADR plus 5-FU combination and ADR plus Ara-C (NSC-63878) were synergistic against P388. ADR plus 5-azacytidine (NSC-102816) was also synergistic against P388. The differential response of P388 and L1210 was probably a consequence of the greater sensitivity of P388 to therapy with many drugs which are only moderately active against L1210. From these and other studies, it is becoming increasingly clear that the best likelihood of observing synergism with two antitumor drugs occurs when they are tested in a system that is quite sensitive to both of the individual drugs. If the main thrust of our efforts is to be the elucidation of the influence of treatment schedule and sequence on the activity of a combination already destined for clinical trial, it is necessary that a system responsive to the combination be identified. On the other hand, the manifestation of potential synergism may be impossible if one or both of the individual drugs is curative. A number of studies have shown that this latter drawback can be overcome by delaying treatment. Thus, although the early P388 tumor is quite sensitive to all of the drugs used in the study, appropriate staging of the disease resulted in the failure of the individual drugs to reproducibly cure the mice while each combination provided a moderate to high percentage of cures. This point was emphasized further by a pilot study conducted at Arthur D. Little in which an attempt was made to determine the influence of sequence of treatment on the effectiveness of ADR plus Cyclophosphamide (NSC-26271) against L1210. ADR plus Cyclophosphamide were known to be synergistic against early L1210 on a variety of concomitant treatment schedules. The plan of the sequential treatment experiment required that one Cyclophosphamide treatment be given on various days ranging from five days before to five days after a single ADR treatment. Synergism was observed only in mice treated with ADR on day 1 and Cyclophosphamide on day 5. There was no synergism when the ADR was delayed, but this was probably due to the ineffectiveness of ADR against the advanced tumor. In retrospect, a tumor very sensitive to both ADR and Cyclophosphamide might have provided a more appropriate system. We plan to extend this type of experimental design to the P388 system.

The group at the Southern Research Institute has developed the established Ridgway osteogenic sarcoma (ROS) for use as a model to evaluate drug combinations of interest. Complete regression of the established tumor and a moderate cure rate among the mice is produced by Actinomycin D (NSC-3053), Cyclophosphamide and Melphalan. Drugs that produce complete regression but no cures include ADR, Daunomycin (NSC-82151), Bleomycin (NSC-125066), VCR, 5-FU, and Palmo-Ara-C (NSC-135962). The disease can be staged so that none of the individual drugs are curative or produce complete regressions and under those circumstances both complete regression and cures were obtained with Act. D plus Cyclophosphamide. ROS appears to be quite suitable for investigations of the influence of schedule and sequence on the efficacy of active combinations.

With respect to the disease oriented approach to the evaluation of combination chemotherapy, additional drugs reported as active against human breast carcinoma were combined against L1210. Clear synergism was observed for Cyclophosphamide plus Thio-TEPA (NSC-6396) or Melphalan. The major thrust of disease oriented combination studies has been the further development of slow-growing tumors as models for human disease. The group at Southern Research Institute developed and evaluated "early passaged" C3H mammary carcinoma for responsiveness to single agents and combinations of interest. Important individual drugs shown to be active in the system were dibromodulcitol (NSC-104800), Cyclophosphamide, the Cyclophosphamide derivative, (NSC-109724), Melphalan, Actinomycin D, Adriamycin, Thio-TEPA, VCR, BCNU (NSC-409962), and Ara-C. On the other hand, 5-FU,Vinblastine (NSC-49842), methyl-CCNU, MTX, and Hydroxyurea (NSC-32065) would not have been detected using this system. Therapeutic improvement over individual drug treatment was observed for ADR plus Cyclophosphamide, Thio-TEPA, or DIC (NSC-45388) and for Thio-TEPA plus Ara-C.

The group at Catholic Medical Center investigated the activity against first generation transplants of CD8F1 mammary carcinoma of 12 drugs reported to be highly effective against human solid tumors and seven which were not. Of the 12 clinically active drugs, Cyclophosphamide, ADR, 5-FU, BCNU, methyl-CCNU, CCNU (NSC-79037), Melphalan, nitrogen mustard (NSC-762), Hexamethylmelamine (NSC-13875), 5-FUdR (NSC-27640), and Thio-TEPA were reported as active in the mice while MTX was inactive. Of the seven drugs regarded as inactive against human solid tumors, only one, Camptothecin sodium (NSC-100880) was active in the mice while Ara-C, 6-MP (NSC-755), L-asparaginase (NSC-109229), picolinoyl-hydrazine (NSC-68626), F3TdR (NSC-75520), and the hormonal agent, NSC-17256E, were inactive. The Catholic Medical Center group also showed Cyclophosphamide plus ADR or 5-FU were more effective than the individual drugs in the CD8F1 system. In addition, the three drug combination of Cyclophosphamide, 5-FU, and ADR was somewhat more effective than ADR plus Cyclophosphamide, the more effective of the two drug combinations. The same group is investigating the metastatic parameters of the spontaneous CD8F1 tumor and the interrelationships between tumor immunity and chemotherapy in anticipation of extending the model for use in chemotherapy plus surgery and chemotherapy plus immunotherapy studies.

The group at Mason Research Institute (MRI), under contract (NO1-CB-50006) sponsored by the Breast Cancer Task Force (BCTF) have developed mammary tumor model test-systems in response to the interests of BCTF. Recently, DEB, through collaborations with the appropriate Project Officer, has extended therapy studies conducted under contract NO1-CM-12259 to these rat models which include the 13762, R-35, and R3230AC mammary adenocarcinomas. All Three tumors are sensitive to radiation therapy. The responses of these tumors to individual drugs will be found in the contractors report to the BCTF. Of significance were the observations that the combination of ADR plus Cyclophosphamide was not more effective than Cyclophosphamide alone against 13762, in contrast to findings with most animal tumor models. However, the combinations of ADR plus 5-FU or MTX appeared to be more effective than the most effective of the individual drugs against 13762.

Within the last year, there has been an effort to utilize existing contractors to evaluate combined modalities of treatment. Southern Research group has demonstrated a therapeutic advantage for surgery followed by BCNU or Cyclophosphamide against the C3H mammary carcinoma; and surgery followed by Cyclophosphamide or methyl-CCNU against LL and subcutaneously implanted B16. Now that appropriate systems have been characterized with respect to the appearence of metastases following tumor implantation, and in view of findings that small metastatic lesions, at any point in time, show a higher proliferative fraction than the large primary solid tumor mass, it appears that a concerted effort to evaluate surgery plus chemotherapy should be made using those systems. We have little information on the influence of the sequence of surgery and drug treatment. Similarly, we should evaluate the effect of cell cycle stage specific treatment on microscopic metastic lesions following removal of large, non-responsive, primary tumors. The same considerations apply to the evaluation of chemotherapy plus radiation or immunotherapy. With respect to chemotherapy plus radiation, DEB expects to participate fully in the workshops anticipated under the guidance of the Deputy Director, DCT and the Associate Director for Cancer Therapy Evaluation. The group at Arthur D. Little has reported that the antitumor activity of MTX, nitrogen mustard, 5 FU, cyclophosphamide, Vinblastine, Ara-C, VCR, Procarbazine (NSC-77213), Actinomycin D, Daunomycin, and Bleomycin was enhanced when the drugs were given in combination with radiation against ip or iv implanted P388. No therapeutic advantage was observed with Prednisone (NSC-10023E) plus radiation. Single dose gamma-radiation to the leg of mice bearing intramuscularly implanted B16 demonstrated the sensitivity of the B16 tumor as evidenced by both tumor inhibition and increases in lifespan at tolerated doses. Combination therapy of radiation plus 5-FU in the IM-B16 system showed slight enhancement of therapeutic effect with respect to tumor inhibition but was no more effective than radiation alone with respect to survival.

During the next year, DEB anticipates development of model systems for the evaluation of chemotherapy plus immunotherapy through collaboration with the Immuno-chemotherapy Section, Laboratory of Experimental Chemotherapy, DR&D.

A major task of the newly formed Molecular Biology and Methods Development Section over the next year will be to collaborate with appropriate DCT investigators and the Deputy Director, DCT to coordinate and critically review projects devoted to studies of tumor and normal cell kinetics and the effects of drugs thereon. The importance of the proliferative state of a tumor cell population to its response to drugs has been well established and the therapeutic activity of a number of important antitumor drugs has been related, in part at least, to their degree of cell cycle specificity of action. Consequently, a number of projects, sponsored by DEB and other DCT units, have been initiated to study these effects. There has been increased emphasis on the investigation of comparative effects on tumor cells and normal cells; e.g., gut crypt cells and hematopoietic cells. There have been substantial technological advances in the rapid measurement of drug effects on single cell suspensions of relatively fast-growing tumors. The question remains as to the applicability of existing methods to the investigation of drug effects on slow growing solid tumors and on metastatic lesions that appear to show a different kinetic picture. Also, we are faced with the question of the translation of in vitro or in vivo experimental studies of cell kinetics and relationships to drug sensitivity to the clinical situation. Specific related questions include (a) the predictive value of the tumors actually being used for experimental kinetic studies and (b) the extent to which existing technology will permit the quantitative measurement of the growth kinetics of human tumors before and after treatment. It is the DEB position that the elegant experimental work in growth kinetics over the past decade has taught us a great many biological principles that affect drug sensitivity, and that program should now make a concerted effort to apply these principles to the individual patient. While the relative proliferative state of the tumor cell population and the critical normal cell population is one important factor that might influence the antitumor specificity of a drug, its treatment schedule dependency, and the efficacy of drug combinations and combined modalities, another is the pharmacokinetics of individual drugs as well as the pharmacological and biochemical interactions of drug combinations. The MBMD Section will collaborate closely with the Experimental Therapeutics Program Area to integrate findings with respect to the influence of cellular and pharmacokinetics on therapeutic efficacy.

The Biochemistry Section (DEB) continued to monitor contract activities related to the biochemical sites of action of active antitumor drugs. A major objective of such studies is to identify new sites of inhibition which may be responsible for the therapeutic efficacy of new structural types and thus form a basis for novel areas of further synthesis. However, within the past year, the Biochemistry Section has been assigned a major share of the responsibility for selection of potential clinical candidates from among the many experimentally active structural congeners of known clinically active drugs. Establishment of our current three-staged screen requires that novel structures with confirmed activity in either the L1210 or P388 initial screen be evaluated further against the slower growing B16 and LL tumors. However, active structural congeners of clinically useful drugs which are very active in in vivo screens may not show sufficiently superior activity to indicate that they should be developed to clinical trial. Moreover, similarity in structure to a clinically active drug may not result in an identical or analogous biochemical or pharmacological action for the new congener. In pursuit of the major task of identifying congeners of known active drugs that might be worthy of development to clinical trial, the Biochemistry Section must collaborate closely with the Associate Director for Cancer Therapy Evaluation, and Associate Director for Medical Oncology, and the Chief, Drug Development Branch to identify those structural types of priority clinical interest. Most of these agents are synthesized under contracts monitored by the Drug Development Branch. However, many are submitted to program voluntarily from diverse sources. One of the critical needs in DR&D is to establish a precise mechanism for rapid identification of the latter. DEB has succeeded in establishing a contract project for the rapid screening of contractor synthesized congeners of active drugs and the direct submission of results to the synthetic chemist as an aid for directing further syntheses. The expanded functions of the Biochemistry Section will be to collaborate with appropriate DCT units to determine protocols for the further evaluation of active congeners on the bases of the biological, biochemical, and pharmacological characteristics as well as the undesirable characteristics of the parent drug of interest; to conduct appropriate studies under contracts initiated and monitored within the section; to collaborate with other DCT units to establish mechanisms for other appropriate studies; and on the basis of integrated results, to recommend superior congeners for development to clinical trial. Such a recommendation may be based on results showing that the active congener is sufficiently different from the parent drug in some important aspect to be considered as a unique active agent or on results showing that the congener, while a true analog of the parent, is superior in some important way.

The major responsibility of Automated Information Section (AIS) is to provide biological data processing services to DCT through appropriate contracts. Major AIS tasks include the computer processing of screening data; dissemination of results to staff, contractors, and suppliers; provision of statistical data to aid in evaluating biological test systems; identification of materials appropriate for further drug evaluation; provision of summary screening data in response to specific queries; and provision of statistical information needed for program management. Major AIS accomplishments during the last year included the computer processing of more than 300,000 test lines of screening data and more than 13,000 test lines of combination chemotherapy data. The latter represented the successful culmination of a concerted effort to establish procedures and mechanisms for the automatic processing of the rapidly increasing body of combination chemotherapy results. Overall, the amount of test data requiring computer processing was nearly double the amount processed in 1972 due mainly to the greatly enhanced input of materials into the primary screen.

Future Course of DEB Projects

DEB anticipates continuation of its screening program utilizing model systems of established predictive value for the clinic. While there is need for new clinically active antitumor drugs, it is clear that substantial improvements in the treatment of human cancer have resulted from the more effective use of older established drugs, and we plan to emphasize studies aimed at providing the clinician with information leading to the design of more effective protocols for clinical use. Such studies, whether they involve empirical *in vivo* testing, cell kinetics and the effects of drugs thereon, or biochemical sites of drug action will be directed to describing optimal therapeutic regimens for active drugs, potentially superior drug combinations and combined treatment modalities and the most effective way to utilize combined therapy. DEB plans to continue to investigate and develop new and improved methods for drug evaluation.

Table 1
Distribution of DEB Monitored Contract Projects by DCT-Array[1/]

Array Code	Linear L	Supplementary S	Exploratory E	Total
		(Amount, $)		
1XX	1,069,230	0	100,900	1,170,130
2AX	4,116,020	45,240	426,470	4,587,730
2B2	1,816,890	2,038,540	713,040	4,568,470
8XX	38,000	0	0	38,000

	(Project)	(Amount, $)
L-1XX	Bioassay *in vitro*	350,670
L-1XX	Bioassay - *in vivo*	577,520
E-1XX	Bioassay - *in vitro* - New assay development	43,900
E-1XX	Biochemical tests aimed at new agent synthesis	57,000
L-2AX	Screening - *in vitro*	388,330
L-2AX	Screening - *in vivo* - Current systems (L1210, P388, B16, LL)	3,162,210
S-2AX	Screening - *in vivo* - Other Systems	29,240
S-2AX	Screening - Biochemical	16,000
E-2AX	Screening - *in vitro* and biochemical - New assay development	153,540
E-2AX	Screening - *in vivo* - New assay development	272,930

(Table 1 Cont'd.)

Distribution of DEB Monitored Contract Projects by DCT-Array[1/]

	(Project)	(Amount, $)
L-2B2	Detailed drug evaluation - *in vivo* (current systems)[*]	1,609,410
S-2B2	Detailed drug evaluation - *in vivo* (established, non-current systems)[*], [‡]	1,249,440
S-2B2	Detailed drug evaluation - *in vitro*[†]	377,000
S-2B2	Detailed drug evaluation - biochemical mechanisms	412,100
E-2B2	Detailed drug evaluation - *in vitro* - Assay development[†]	28,500
E-2B2	Detailed drug evaluation - *in vivo* - Assay development[*], [†], [‡]	684,540
L-1XX	Tumor Bank	8,400
L-2AX	Tumor Bank	21,000
L-2B2	Tumor Bank	12,600
L-1XX	Automatic data processing	132,640
L-2AX	Automatic data processing	544,480
L-2B2	Automatic data processing	194,880
L-8XX	Administrative and information function	38,000
Total -	DEB Monitored projects	10,364,330

[*] Includes combination chemotherapy and combined modality treatment.

[†] Includes tumor cell kinetics and effects of drugs thereon.

[‡] Includes use and development of animal models of human tumors.

Table 2
DEB Contractors. Distribution of Effort Among Projects

CONTRACTOR (Contract Number) Projects Monitored by Drug Evaluation Branch	Funding of Tasks Monitored by Drug Evaluation Branch Distribution by Project		
	(Code)	(%)	($)
ALLEGHENY GENERAL HOSPITAL - 43730		100	79,000
Detailed drug evaluation - in vitro	S-2B2	50.0	39,500
Detailed drug evaluation - in vivo	S-2B2	50.0	39,500
ATOMIC ENERGY COMMISSION - 10056		100	25,000
Detailed drug evaluation - in vitro	S-2B2	70.0	17,500
Related new assay development	E-2B2	30.0	7,500
BATTELLE MEMORIAL INSTITUTE - 12182		100	494,000
Bioassay - in vivo	L-1XX	1.0	4,940
Screening - in vivo	L-2AX	79.0	390,260
Detailed drug evaluation - in vivo	L-2B2	20.0	98,800
BRISTOL - MYERS CO. - 90035 *		50	228,500 457,000
Bioassay - in vitro	L-1XX	15.0	68,550
Bioassay - in vivo	L-1XX	20.0	91,400
Screening - in vitro	L-2AX	4.0	18,280
Screening - in vivo	L-2AX	11.0	50,270
CATHOLIC MEDICAL CENTER - 23703		100	342,000
Detailed drug evaluation - in vivo	S-2B2	90.0	307,800
Related new assay development	E-2B2	10.0	34,200
DELAWARE, UNIV. OF - 92115		100	0
Enzyme production	L-1XX	100	(Terminated)

Table 2 (Cont'd.)
DEB Contractors. Distribution of Effort Among Projects

HAZLETON LABORATORIES - 23704		100	731,000
Bioassay - *in vivo*	L-1XX	14.0	102,340
Screening - *in vivo*	L-2AX	56.0	409,360
Screening - *in vivo*	S-2AX	4.0	29,240
Detailed drug evaluation - *in vivo*	L-2B2	21.0	153,510
Detailed drug evaluation - *in vivo*	S-2B2	5.0	36,550
IIT RESEARCH INST. - 43755 (Screening Services)		100	116,000
Bioassay - *in vitro*	L-1XX	7.0	8,120
Bioassay - *in vivo*	L-1XX	13.0	15,080
Screening - *in vitro*	L-2AX	8.0	9,280
Screening - *in vivo*	L-2AX	72.0	83,520
IIT RESEARCH INST. - 81021		84	840,000 1,000,000
Bioassay - *in vivo*	L-1XX	10.0	100,000
Screening - *in vivo*	L-2AX	41.0	410,000
Related assay development	E-2AX	4.0	40,000
Detailed drug evaluation - *in vivo*	L-2B2	20.0	200,000
Detailed drug evaluation - *in vivo*	S-2B2	9.0	90,000
INST. JULES BORDET - 22053*		25	10,000 40,000
Screening - *in vivo*	L-2AX	20.0	8,000
Detailed drug evaluation - *in vivo*	L-2B2	5.0	2,000
JAPANESE FDN. CANCER RESEARCH - 22054*		25	13,750 55,000
Screening - *in vivo*	L-2AX	25.0	13,750

Table 2 (Cont'd.)
DEB Contractors. Distribution of Effort Among Projects

LITTLE, ARTHUR D. - 33727 *		59	1,239,000 2,100,000
Bioassay - *in vitro*	L-1XX	4.0	84,000
Related new assay development	E-1XX	2.0	42,000
Bioassay - *in vivo*	L-1XX	0.5	10,500
Screening - *in vitro*	L-2AX	1.0	21,000
Screening - *in vivo*	L-2AX	23.0	483,000
Related new assay development	E-2AX	2.0	42,000
Detailed drug evaluation - *in vitro*	S-2B2	1.5	31,500
Related new assay development	E-2B2	1.0	21,000
Detailed drug evaluation - *in vivo*	L-2B2	13.0	273,000
Detailed drug evaluation - *in vivo*	S-2B2	7.0	147,000
Related new assay development	E-2B2	2.0	42,000
Tumor bank	L-1XX	0.4	8,400
Tumor bank	L-2AX	1.0	21,000
Tumor bank	L-2B2	0.6	12,600

LITTLE, ARTHUR D. - 23257 (ADP improvements)		100	50,000
Bioassay - *in vitro*	L-1XX	7.0	4,200
Bioassay - *in vivo*	L-1XX	13.0	7,800
Screening - *in vitro*	L-2AX	8.0	4,800
Screening - *in vivo*	L-2AX	72.0	43,200

LITTON BIONETICS - 33741		100	320,000
New assay development - *in vitro* and biochemical	E-2AX	30.0	96,000
Detailed drug evaluation - *in vitro* and biochemical	S-2B2	70.0	224,000

MARIO NEGRI INST. - 33720		85	126,650 149,000
Screening - *in vivo*		85.0	126,650

MASON RESEARCH INST. - 12259		100	631,000
Bioassay - *in vivo*	L-1XX	2.0	12,620
Screening - *in vivo*	L-2AX	60.0	378,600
Related new assay development	E-2AX	3.0	18,930
Detailed drug evaluation - *in vivo*	L-2B2	30.0	189,300
Detailed drug evaluation - *in vivo*	S-2B2	5.0	31,550

Table 2 (Cont'd.)
DEB Contractors. Distribution of Effort Among Projects

MIAMI, UNIV. OF - 02225		100	154,000
Bioassay - *in vitro*	L-1XX	2.0	3,080
Screening - *in vitro*	L-2AX	98.0	150,920
MICHIGAN DEPARTMENT HEALTH - 33702		100	48,000
Bioassay - *in vitro*	L-1XX	100	48,000
MICROBIOLOGICAL ASSOCIATES - 33728*		40	556,800 1,392,000
Screening - *in vivo*	L-2AX	13.0	180,960
Detailed drug evaluation - *in vivo*	L-2B2	13.0	180,960
Detailed drug evaluation - *in vivo* - drug metabolism and distribution	S-2B2	12.0	167,040
New assay development	E-2B2	2.0	27,840
PENNSYLVANIA, UNIV. OF - 12188		100	13,000
Screening - biochemical - new assay development	E-2AX	30.0	3,900
Detailed drug evaluation - biochemical mechanisms	S-2B2	70.0	9,100
RESEARCH TRIANGLE INST. - 92019*		15	20,550 137,000
Bioassay - *in vitro*	L-1XX	15	20,550
SCRIPPS CLINIC - 22042		100	49,000
Bioassay - *in vitro*	L-1XX	75.0	36,750
Screening - *in vitro*	L-2AX	13.0	6,370
Screening - *in vivo*	L-2AX	12.0	5,880
SOUTHERN RESEARCH INST. - 12189		100	0
Screening - biochemical - new assay development	E-2AX	100	(Terminated)

Table 2 (Cont'd.)
DEB Contractors. Distribution of Effort Among Projects

SOUTHERN RESEARCH INST. - 60029		100	403,000
Detailed drug evaluation - Biochemical mechanisms	S-2B2	100	403,000
SOUTHERN RESEARCH INST. - 12098		100	2,150,000
Screening - *in vitro*	L-2AX	6.0	129,000
Screening - *in vivo*	L-2AX	16.0	344,000
Related new assay development	E-2AX	8.0	172,000
Detailed drug evaluation - *in vivo*	L-2B2	20.0	430,000
Detailed drug evaluation - *in vivo*	S-2B2	20.0	430,000
Related new assay development	E-2B2	27.0	580,500
Detailed drug evaluation - *in vitro*	S-2B2	1.0	21,500
Detailed drug evaluation - microbial assay	S-2B2	2.0	43,000
TRACOR JITCO - 33744		100	38,000
Administrative and information fc.	L-8XX	100	38,000
UPJOHN COMPANY - 43753*		32	286,080 894,000
Bioassay - *in vitro*	L-1XX	6.0	53,640
Bioassay - *in vivo*	L-1XX	4.0	35,760
Screening - *in vitro*	L-2AX	6.0	53,640
Related new assay development	E-2AX	6.0	53,640
Screening - *in vivo*	L-2AX	4.0	35,760
Detailed drug evaluation - *in vivo*	L-2B2	6.0	53,640
VALUE ENGINEERING - 33706 (ADP)		100	696,000
Bioassay - *in vitro*	L-1XX	5.0	34,800
Bioassay - *in vivo*	L-1XX	9.0	62,640
Screening - *in vitro*	L-2AX	6.5	45,240
Screening - *in vivo*	L-2AX	51.5	358,440
Detailed drug evaluation - *in vivo*	L-2B2	28.0	194,880

Table 2 (Cont'd.)
DEB Contractos. Distribution of Effort Among Projects

WARF INST. - 60494		100	564,000
Bioassay - *in vivo*	L-1XX	39.0	219,960
Screening - *in vivo*	L-2AX	56.0	315,840
Detailed drug evaluation - *in vivo*	L-2B2	5.0	28,200
WISCONSIN, UNIV. OF - 33733		100	38,000
Bioassay - *in vitro*	L-1XX	95.0	36,100
Related new assay development	E-1XX	5.0	1,900
YALE UNIV. - 33711		67	16,000 24,000
Screening - biochemical	S-2AX	67.0	16,000
YALE UNIV. - 12339		100	76,000
Biochemical tests aimed at new agent synthesis	E-1XX	75.0	57,000
Screening - *in vitro*	L-2AX	12.0	9,120
Screening - *in vivo*	L-2AX	13.0	9,880
CENTRAL DRUG RES. (INDIA) - PL-480*		25	---
Bioassay - *in vivo*	L-1XX	15.0	---
Screening - *in vivo*	L-2AX	10.0	---
WEIZMANN INST. (ISRAEL) - PL-480		50	---
Screening - Biochemical - Assay development	E-2AX	50.0	---
WEIZMANN INST. (ISRAEL) - PL-480 *		20	---
Screening - *in vivo*	L-2AX	20.0	---

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* Contract sponsored by DCT unit other than DEB. For contracts in which only a part of the total effort is monitored by DEB, the percentage of effort monitored by DEB is listed and funding is shown as the amount monitored by DEB/total cost.

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Automated Information Section
Drug Evaluation Branch

The objective of the Automated Information Section is to provide biological data processing services to the Division of Cancer Treatment with major responsibility for implementation and maintenance of the biological data processing system for the Drug Evaluation Branch. New agents enter the system when they are shipped to laboratories for testing. The resulting screening data from contract and DR&D laboratories are processed and disseminated to staff, contractors and suppliers. The system provides statistical data to assist in the evaluation of both the primary screen and new test systems and is utilized to identify materials for further testing as well as those worthy of consideration for DCT Decision Point 2A. It is queried by special programs to provide summary screening data to assist in the further evaluation of materials as anticancer agents. It also provides statistical information needed for program management.

The current data processing contractor has had a contract since July 1972 for the operation of the biological data processing system. At the beginning of the calendar year, all programs received from the previous contractor had been revised so that they could be run at DCRT. During 1973, 14,441 experiments, which include 385,054 single drug tests and 13,628 drug combination tests were processed; an increase of 16% over 1972. This produced 395,152 single drug tests and these are shown by test type in Table 1. A total of 43,770 materials were added to the master files; an increase of 48% over 1972.

Costs (both contractor and DCRT computer costs) were reduced from $2.15 per test in 1972 to $1.90 per test in 1973 due to increased program efficiencies and increased efficiencies in data handling such as bi-weekly production runs.

The following reports were produced and distributed routinely, schedules being interrupted only for modifications to the combination chemotherapy data processing program:

Routine System Output:

- Screening Data Summaries - about 7,200 were mailed to suppliers and screeners on a bi-weekly schedule
- Screening Experimental Analysis Reports
- Interim Screening Transaction Reports
- Address labels for mailing
- Combination Chemotherapy Reports

DR&D Program Management Aids:

- Automated Schedules (Quarterly)
- Control Pack Index - index to microfilm of raw test data
- Material Shipping List Index - index to microfilm of raw shipping data
- Quarterly Statistical Report
- Forms Control Report
- Combination Chemotherapy Cross Reference Report
- Selected Agents Compounds (SAC) Report

DEB, Screening Program Management Aids:

- Error Analysis Report
- Control Performance Report
- Production Report
- Control Line Listing
- Control Performance Summary Report
- Bi-Weekly Statistical Report by Special Study Code
- Missing Control Pack Report
- Schedule Dependency Report
- Status Code 15, 26P, 27P, 28P, 31P reports (Confirmed Active Testing)

DDB, Natural Products Management Aids:

- Materials of Interest Report (Natural Products)
- Confirmed Plant and Animal Report
- Plant Header Reports (Botanical Names)
- New Plant Extracts (Fractionator)
- Quarterly Fractionation Report
- Cross Index to Plant Header File

The synthetic Master File was microfilmed in September, 1973. In addition, the Quarterly Plant Header Reports were changed from output on paper to output on microfilm.

New automated reports were developed to assist in management of the program. These include an automated Systems Statistical Report which summarizes the many activities of the Drug Research and Development Program. In addition, the Production Report, which was formerly prepared manually, was automated to summarize in vivo, in vitro test data, toxicity data, alkaloid and biochemical assays, and the drug combination program data. This report is produced quarterly and contains cumulative year-to-date totals by test system, laboratory and type of material. An automated Error Analysis Report was developed to show the type of errors being encountered by each screener.

The number of combination studies processed continued to increase this year. In addition to processing the test data, a cumulative cross reference index is produced with each update of the file. It now includes the chemical names of the compounds, as well as the NSC number and indicates which compounds had new data added in the most recent update. The drug combination program was modified to update the calculations for median survival time, mean survival time, and eliminate printing toxic T/C values. The updated median survival calculations were also implemented in the other appropriate programs.

Efforts were begun by the data processing contractor to redesign the system to simplify the edit and calculation programs which will result in greatly reduced maintenance costs for Pre-Validity, Validity, Cell Culture, and Combination programs.

Efforts were made to begin handling data produced on line by screeners using terminals. At the end of calendar year 1973, the data processing contractor was receiving and successfully processing data from one screening laboratory sent on magnetic tape. In parallel, procedures were being developed to accept data directly from an on-site terminal at the screener's facility.

In addition to the regularly scheduled reports listed previously, a number of reports are produced as requested. A major portion of staff time is utilized by providing these special reports to contractors, suppliers, and DCT staff members. Numerous programs were written to summarize screening test results to fit the specific needs of individual staff members in the evaluation of the data. During the year over 75 special queries were processed. These include staff requests to retrieve materials by a number of different identifiers, such as tests systems utilized, supplier, and material classification.

Two programs have been written and are now routinely produced to assist the new Screening Services Contractor; one summary report facilitates the scheduling of testing for refills or duplicates of materials received previously, and the second program reports the data on all synthetic materials updated in most recent production run. This report enables the contractor to assist the Screening Section, DEB in the evaluation of the test results of synthetic materials.

The schedule dependency summary and plotting program files have continued to be updated by AIS staff. Approximately 100 schedule dependency experiments have been summarized and added to the file this year.

To support increasing requests from the Department of Agriculture for information on collected plant and animal materials, a plan was implemented where queries were supplied on indexed microfiche rather than paper. This resulted in large savings in cost, storage space, and handling. Information has also been supplied to others on microfilm or microfiche where practical. For example, a listing, by original supplier was produced for the Scientific Records Section, DDB from our synthetic files and identified all materials which each supplier had submitted.

During this year this section monitored a contract with the A.D. Little, Co. Inc., for the review and analysis of our data processing system. One of the goals of this contract was to provide a reporting form more useful to our suppliers. A brief English message reporting the status of the testing was added to the Screening Data Summary form this year.

The Automated Information Section is composed of a staff of seven; 2 professionals, 1 junior professional, 1 statistical assistant, 1 computer aide, 1 project Stride trainee, and 1 secretary. Staff serves as Project Officers and has responsibility for three contracts: Value Engineering Co., Contract No. N01-CM-33706, which provides the major service for the biological data processing system; Tracor Jitco, Contract No. N01-CM-33744, which maintains a chemical drug distribution and inventory system; and A.D. Little, Inc., Contract No. N01-CM-23257 for analysis and recommendations for improvement of our data processing system.

Table I

PROGRAM STATISTICS

1. Materials Added to the Master Files[1]	1973	1972
Synthetic Compounds	26,906	14,781
Selected Agents	214	--
Plants	11,066	10,434
Fermentation products	2,550	2,712
Animal Products	3,034	1,669
Total	43,770	29,596
2. Tests Performed		
L1210 Lymphoid Leukemia[2]	134,562	103,681
P388 Lymphocytic Leukemia	198,723	180,027
AKR Leukemia	2,049	4,501
Walker Carcinosarcoma	1,704	2,770
B16 Melanoma	15,989	6,028
Lewis Lung Carcinoma	2,441	4,141
Other Tumors	647	1,957
Total In Vivo (excluding toxicity)	356,115	303,105
Toxicity	6,430	2,900
Cell Culture	31,820	25,996
Alkaloid	450	--
Biochemical Assay	337	231
Total	395,152	332,232

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1. These figures are derived from the Drug Research and Development Biological Data Processing System and differ from those generated by the Drug Development Branch, which logs in materials when NSC numbers are assigned.

2. These figures include tests against L1210 resistant test systems.

BIOCHEMISTRY SECTION

DRUG EVALUATION BRANCH

DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

The ultimate objective of the Biochemistry Section program is the improved therapy of human cancer by drugs. The program is implemented through contracts with qualified institutions and through informal, unfunded arrangements with individual investigators, usually at universities, who have produced agents of theoretically exploitable qualities which require special testing to demonstrate anticancer activity, or who possess mastery of particular techniques and procedures needed for definitive work on DRD drugs. The following describes first the work being done under contract, and next that under the informal arrangements.

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NO1-CM-22042)

During the first year this contract was in operation, a series of about a dozen B_{12} coenzyme analogs were prepared with unexpected ease. Then followed, during this year, equally unexpected difficulties in assessment of the utility of the agents as possible antitumor agents. Much of the problem encountered in evaluation has probably been due to lack of basic information in the area of transport requirements and actual metabolic targets in mammalian cells. Hence the filling in of some scientific background preparatory to straightforeward assessment of the agents has constituted a large part of the work of the present contract year.

Both B_{12} and its analogs have been shown to be transported into eukaryotic cells by a serum protein, transcobalamin II (TC-II). To test the antitumor activity of the analogs in vitro an adequate amount of free TC II must be present in the media. Because calf serum has no unbound TC II, this protein must come from either another source of serum, or by added TC II. Purified TC II is also necessary to assess the analogs as inhibitors of B_{12} transport. To evaluate the analogs in these two systems, preparation of the transport protein has been undertaken.

Using the purified apoenzyme of ribonucleotide reductase from Lactobacillus leichmannii, the analogs have been screened as competitive inhibitors of natural adenosyl-B_{12}. Two of the analogs, AraA-B_{12} and formycinyl-B_{12} have sufficiently good K_i values (ca. 10^{-6} M) to make them candidates for testing as antitumor agents. Conclusions reached from enzymatic results to date indicate: (1) that B_{12} enzyme systems have rather rigid structural requirements with respect to both base and sugar moieties, so that analogs which are quite different are not likely to be effective inhibitors, and (2) that because most, if not all, of the B_{12} systems in the cell have tightly bound coenzyme analog or natural B_{12}, they are not likely to replace each other. Hence it might require a B_{12} analog to be present over several cell generations to be effectively cytotoxic. However, fortunately, a rather large part of the B_{12}-requiring enzyme, methionine synthetase, is present in L1210 cells as the apoenzyme, and mouse serum, like that from normal humans, has a fair amount of unbound TC-II.

SOUTHERN RESEARCH INSTITUTE (NO1-CM-12189)

The objective of this contract is to establish whether there are anticancer agents which exert cytotoxic action primarily as a result of inhibition of membrane formation. The approach chosen was to determine *in vitro* the effect of an agent on cell growth and on incorporation of labeled precursors into membrane constituents and DNA. The most-studied precursor for membranes has been choline, with lesser effort to date on glucosamine, serine, acetate, inositol, etc. While Ca755 cells were used initially, they were rather soon replaced by L1210 cells. More than 40 agents, most of which have some antitumor activity, have been evaluated, of which six have exhibited preferential inhibition of one or more membrane precursor over TdR into DNA or over cell growth.

The real question in determining the value of this contract was what the level of preferential inhibition (membrane over cell replication) must be when this becomes the prime cause of cell stasis or death; or, indeed, whether such an inhibitory mechanism can in itself be lethal. To attempt an answer to this basic question, the Drug Development Branch was asked to prepare tetrolylpantetheine (NSC-169437) and four variations (NSC-166364, 166906, 167679 and 169436). NSC-169437 was found by Dr. R. O. Brady to inhibit the incorporation of acetate-1-^{14}C into the lipids of rat skin by 70%. The quantity of the actual "Brady compound" (NSC-169437) was too small to permit *in vivo* testing, and too non-toxic (insoluble?) for *in vitro* results. None of the other agents showed selective inhibition of choline over thymidine incorporation, nor did they have *in vivo* antitumor activity.

After evaluation of the data accumulated to the time of consideration of renewal of this contract, the reviewing committee felt that the objective was not being achieved and recommended extension for a six-month phase-out period. Accordingly, this contract was terminated December 16, 1973.

SOUTHERN RESEARCH INSTITUTE (NO1-CM-60029)

This is a multifaceted contract in the general area of biochemistry, with the overall aim of advancing chemotherapeutic control of cancer. A small part of the work is devoted to the drug synthesis contract with the same institute (contract NO1-CM-33712). The Biochemistry contract suggests agents for synthesis, and tests them in appropriate ways (other than *in vivo* screening) for possible utility as anticancer agents.

Another part of the work (~ 44%) under this contract is done at the direct request of the NCI. Usually this is to define, if possible, the mechanism of action of agents well along the path toward clinical trial, about which nothing is known. Among such agents are: 3-deazauridine (NSC-126849), a dialdehyde of inosine (Inox, NSC-118994), and a tricyclic 7-deaza nucleoside (NSC-154020).

Another type of request by NCI, is to "sift" all *in vivo*-active purine bases and nucleosides acquired by NCI in a spectrum of H.Ep.#2 cell lines of known enzyme deletions. This serves as a first step to separate the unusual agent which is biologically different from those classified on a structural basis as analogs of a well-known parent compound.

A third type of work is contractor-generated, growing out of the investigators' long-time interests and observations. Projects falling into this area are study of: ribonucleotide reductase inhibitors, development of a screen for agents inhibiting DNA repair, and various aspects of the mechanism of action of nitrosoureas, of 6-thioguanine, and of resistance to 6MP.

Findings this year on these projects are as follows:

Work on Synthesis Contract Compounds:
During the year more than fifty new compounds synthesized by the Organic Chemistry Department of SRI were screened for appropriate activity. These embraced a wide variety of structures, including 2-azapurines and nucleosides, 8-azapurines and nucleosides, analogs of methylthioadenosine, carbocyclic analogs of nucleosides, deazapteridines, cytosine derivatives, and imidazoles. One, and possibly two agents may be of interest. The first is 8-aza-6-MPR (NSC-175374) which on one test at 50 mg/kg on day 1 only, cured 2/6 L1210 mice and produced an ILS of 66% in the remaining mice; no activity at all was shown on a second test. It is much less toxic *in vitro* than 6MP or MeMPR, is poorly cleaved to free base, and is activated by a kinase. The second agent, a deazapteridine (NSC-128300) had high *in vitro* cytotoxicity, ED$_{50}$ ~ 0.01 μg but does not inhibit dihydrofolate reductase. *In vivo*, the agent gives a T/C of 125-130 at 25 mg/kg/d x 9.

3-Deazauridine (NSC-126849):
The major effect of this agent is to decrease CTP synthesis as shown by inhibition of ^{14}C-orotic acid into cytidine nucleotides and in markedly decreased cellular pools of CTP and dCTP. AraC-resistant L1210 cells were particularly sensitive to 3-deazauridine because they cannot utilize exogenous dCTP to circumvent drug-inhibited endogenous production of it.

Inox(NSC-118994):
The earliest effect of this agent on L1210 cells in culture was inhibition within two hours of RNA synthesis. By 48 hours, synthesis of DNA and protein was also inhibited, probably as a secondary event. Incorporation of ^{14}C-formate into azaserine-induced accumulation of formyl-glycineamide ribonucleotide (FGAR) was not reduced by Inox, thus indicating that the agent does not act as a feedback-type inhibitor.

"Tricyclic Nucleoside" (NSC-154020):
This agent is closely related structurally to sangivamycin, although the third ring, which extends from the 6-position to the 7-deaza of purine, is said to be stable; not opening to give a sangivamycin derivative. Comparative studies of NSC-154020 and sangivamycin on the incorporation of labeled formate, hypo-

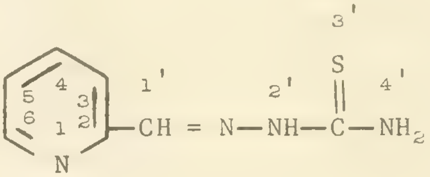
xanthine, and leucine by L1210 cells in culture revealed no differences between the two agents; both inhibited the utilization of the three precursors. Both agents were easily phosphorylated by adenosine kinase and the phosphate is necessary for cytotoxicity of both.

Screening of Purines and Nucleosides Against a Spectrum of H.Ep.#2 Cells in Culture:

As stated earlier, the purpose of this screen is to select compounds that appeared to be metabolically activated by unexpected mechanisms or by a mechanism different from that of structurally related compounds. The agents so selected should be candidates for further study and evaluation to make them ready for a decision on their potential as future clinical drugs. A few agents are emerging as interesting.

Inhibitors of Ribonucleotide Reductase:

In a continuation of structure-activity study, constituting about 2% of this contract, of inhibitors of human ribonucleotide reductase, the effects of alkyl substituents at various positions of 2-formylpyridine thiosemicarbazone were determined. The following table gives the data.



Compound	Concentration for 50% inhibition of ribonucleotide reductase activity (Molar)
1. Unsubstituted (NSC-729)	2.8×10^{-7}
2. 1'-Methyl	1.5×10^{-6}
3. 2'-Methyl	5.2×10^{-3}
4. 4'-Methyl	3.0×10^{-6}
5. 4',4'-Dimethyl	1.2×10^{-6}
6. 3-Hydroxy	6.4×10^{-6}
7. 3-Hydroxy-3'-methyl	1.9×10^{-4}
8. 3-Hydroxy-3',4'-dimethyl	8.0×10^{-5}

Mr. F. A. French, who prepared the 4',4'-dimethyl derivative, reports that this agent retains the *in vivo* antitumor activity of the unsubstituted agent against L1210 and Lewis lung tumors.

Inhibitors of DNA Repair:
About 9% of the contract effort has been expended in an attempt to set up a system to screen agents as inhibitors of DNA repair. This is the third year of effort on this project and success still eludes the undertaking. This year two *in vitro* systems were investigated; one based on drug inhibition of the stimulation of DNA synthesis induced by ultraviolet irradiation, and the second based on the survival of cells cultured in media containing the inhibitory candidate and an alkylating agent or exposure to ultraviolet light. Both proved unsatisfactory, the first changes were too small to measure accurately, and the second because caffine, which is widely reported to suppress such repair, showed no such effect.

Nitrosoureas:
Work on the nitrosoureas is the largest of the contractor-generated projects. In itself it accounts for 30% of the contract expenditures. Six items as follows indicate the breadth of study: A. Duration of Inhibition of Synthesis of DNA and RNA in Tumors and Host Tissues after Single Doses of Nitrosoureas. B. Studies Related to Alkylation and Carbamoylation Reactions of Reactive Entities Derived from Nitrosoureas. C. Interrelationships of Some Chemical, Physicochemical, and Biological Activities of Several 1-(2-Haloethyl)-1-nitrosoureas. D. Comparisons of the Half-lives, Alkylating Activities, and Carbamoylating Activities of Several Nitrosoureas Including Some with Deoxyglucosyl Groups on N-3. E. Fixation of ^{14}C by Sensitive and Resistant Bacteria and Mammalian Cells. F. Enzymatic Metabolism of 1,3-Bis(2-chloroethyl)-1-nitrosourea (NSC-409962, BCNU). While much effort went into the work, it has been in general a deeper look and finer polish of previously studied areas. The new data indicated that often the most carefully-made *in vitro* or chemical observations are confounded by new parameters introduced in *in vivo* situations. The one really new attack this year was studies of liver cell metabolism of BCNU, CCNU, and MeCCNU. All three agents were substrate for a TPNH and oxygen requiring microsomal enzyme and for a soluble cellular enzyme requiring no cofactors. In the case of the BCNU the microsomal enzyme produced-product has been identified and determined to have lost antitumor activity, thus serving as a detoxifying mechanism.

Mechanism of Action of 6-Thioguanine (NSC-752):
Studies of the mechanism of action of thioguanine were designed to determine whether there was another in addition to that already-defined one of incorporation into DNA. None has been shown.

Mechanism of Resistance to 6MP (NSC-755):
Work was done to confirm the mechanism of resistance of 6MP reported in the literature for E. coli and human leukocytes; that of the presence of a cellular enzyme to remove sulphur from 6MP, 6MeMPR (NSC-25740) or 6-thio IMP (NSC-4911)

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM-33711)

During this year 22 more antifols have been screened for inhibitory activity of dihydrofolate reductase from human cells.

The physiological disposition of NSC-139105, the Baker Antifol, in laboratory animals (mice, rats, dogs, and monkeys) has been completed and the results published, while that in patients is underway. The effect of NSC-139105 on the incorporation of tritiated UdR into DNA in cancer cells from 16 patients has been done. A greater than 95% inhibition was found with concentrations of 1×10^{-5}M, but none with 2×10^{-6}M. Clinically, Dr. Bertino has worked up to a dose level of 100 mg/M^2/day X 5 in an ALL child without toxicity and with a reduction in blast count from 200,000 to 40,000/mm^3. Serum disappearance of NSC-139105 in dogs and monkeys was similar, with a half-life of 3-4 and 2-4 hours respectively. Urinary excretion at 24 hours was 5-6% of the injected dose in mice and rats, but 60% in dogs. Accumulation of drug were highest in liver and progressively less in kidney spleen and brain. In dogs the CNS level was 15% of the serum concentration at one hour. No evidence of drug metabolism was found in extracts of urine, feces, or organ tissues.

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1-CM-12339)

The objective of this contract is to design and produce new, active anticancer agents. To do this physical-chemical measurements are made of compounds most likely to interfere with the synthesis and other functions of DNA by prevention of strand separation. From knowledge so gained, potentially useful new modifications of parent structures are being designed and synthesized, followed by determination of the physical and biological properties of the new agents.

The physical properties determined are the tightness of binding of agent to nucleic acid and the extent to which the agent prevents strand separation as shown by change in melting temperature (Tm). The biological consequences of alterations in DNA strand separation showed up in changed RNA produced. Delineation of the changes in each of the types of nuclear RNA was done. Finally in vitro and in vivo antitumor activity, which is the ultimate monitor of desirability of the altered transcription and translation brought about by the new agents, was determined.

During the last year the contractor has made and tested a number (upward of 16) of "double intercalators"; agents made up of two acridine molecules, one bound to each end of a linking agent. Although the first series, using the naturally-occurring polyamines putrescine and spermidine as the link, were dramatically effective in increasing the Tm of DNA, they had two biological drawbacks: (a) they were very toxic and (b) they gave erratic in vivo antitumor activity due, as was found, to reversal of binding by spermine.

However, using straight chains of $-CH_2-$ groups as the link, there was decreased toxicity, increased binding stability and improved in vivo antitumor activity (optimal at $(CH_2)_6$or $_8$ lengths).

All of the rapidly labeled forms of RNA (HnRNA, rRNA and mRNA) were changed in base composition and other respects in ways different than those caused by actinomycin D. The latter agent binds to dGdC while the diacridines bind this pair, but also dAdT and polyApolyU.

In vivo testing of actinomycin D and diacridines in combination in P388 gave at least additive antitumor effects.

On theoretical grounds, an anticancer agent can be expected from the type of agent being synthesized. Already agents with acceptable in vivo increases in the life-span of tumor-bearing mice have been produced. The pertinent and sophisticated physical, biochemical, and biological studies, both present and proposed, may well lead to better agents. Studies so far indicate that these "double intercalators" of acridine have a somewhat different spectrum of effects than that of the classical nucleic acid intercalator, actinomycin D.

INFORMAL COLLABORATIONS

DR. KARL FOLKERS, UNIVERSITY OF TEXAS

Dr. Folkers, an organic chemist, is interested in quinones, particularly those which might act as Coenzyme-Q analogs and interfere with mitochondrial electron transport and thus cellular energy production. He has synthesized quite a number of these agents, most in very small quantities ($<$ 25 mg.) and the question was how they should be tested. A few were sent to Dr. Britton Chance at the University of Pennsylvania (Contract NO1-CM-12188). However, in vitro and eventually in vivo biological tests are needed to establish utility in the cancer program.

A few of the earliest agents had been found active by Dr. Folkers for in vivo anticancer activity against Ehrlich ascites tumor. One of the most active of these (NSC-186889) has been supplied in sufficient quantity for us to confirm activity in Ehrlich. Further quantities have now been sent for testing in our current tumors, L1210, P388, B16, and possibly Lewis Lung. The testing in Ehrlich indicated that at responsive doses the agent was without toxicity on prolonged daily treatment and suggested that the agent might be carcinostatic. Accordingly, it is currently under test in P388 and i.p. B16 on a 30-day treatment schedule.

Should the agent be active in L1210 or P388, the testing of the many small amount Coenzyme-Q analogs could be tested in vitro in these tumors to select the agents to make in larger quantities for in vivo testing.

DR. G. A. LEPAGE, UNIVERSITY OF ALBERTA

A Drug Development Branch contractor, Dr. LeRoy Townsend of the University of Utah, has synthesized a considerable number of selenium (for sulfur) analogs of TG and 6MP. Many had considerable in vivo antitumor activity. In order to decide whether or not the best of this series was of interest to develop for the clinic, we needed to know whether the mechanism of action was the same as or different from that of TG.

Dr. LePage does most exquisitely precise biochemical, pharmacological, and in vivo testing studies on agents related to thioguanine.

Accordingly, the problem was discussed with Dr. LePage, who agreed to look into the matter for us. More of selected agents from the group and radioactive Se are being supplied to him for this purpose, and the results, as obtained, will be sent to us.

DR. ARTHUR GROLLMAN, EINSTEIN COLLEGE OF MEDICINE

Dr. Grollman is a pharmacologist whose research is wide-ranging searches for mechanisms of action of drugs. He has often been given (with DRD approval) natural products prepared under contract by Dr. Kupchan of the University of Virginia. In the course of a discussion of some of his results on these agents, Dr. Grollman expressed interest in studying other drugs of interest to DRD. Accordingly the following eleven drugs have been sent to him: Mithramycin (NSC-24559), a pyrazolo-pyrimidine (NSC-51143), Chromomycin A_3 (NSC-58514), Ellipticine (NSC-71795), Tritylcysteine (NSC-83265), MeCCNU (NSC-95441), Baker antifol (NSC-139105), Anguidine (NSC-141537), Homoharringtonin (NSC-141633), and Maytansine (NSC-153858). Some preliminary results on some agents have been communicated by phone, and written reports will be sent when work is complete, followed by reprints when published.

FUTURE PLANS

There are two additional projects which are being undertaken by the section. The first is the one-time task of identifying for further study agents acquired in the past , the antitumor activity of which fell below the previous cut off of T/C = 150 and the level which meets our new screening cut off of T/C = 125. Not all agents with activity between these limits will be candidates for further study; analogs of more active drugs, for instance, will not be. However, all data at our command on agents which are of unique structure (no analogs among our acquisitions) as well as the most active drug of a new class of agents should be scrutinized to decide whether more agent should be secured for further testing. A committee has been set up to assist in the scrutiny of data provided by the section.

The second task is a long-term and slow-yielding one. It is to identify among active analogs of well-known agents, those which possess some important property which would make them worthy clinical candidates. In dealing with this problem many aspects need to be considered, and each series of drugs will need to be dealt with in a different fashion. Varying scientific expertise will also be required, sometimes to be found in contracts within the section and branch, often extending to those in other parts of the Division of Cancer Treatment or to new resources outside the division or even NIH. Governing all selection of classes of agents to receive this kind of work would be clinical interest and/or clinical problems. An example or two may help in clarification.

Adriamycin and related drugs have a cardiotoxicity which is clinically dose-limiting. In 1972 de Duve and collegues published their finding that daunorubicin, which, like all members of this class of drugs, intercalates into DNA, when bound to DNA, was less toxic and gave greater antitumor effects in

L1210 bearing mice. The combination of adriamycin and DNA was prepared and tested under Dr. Venditti's direction. The reported finding of decreased toxicity and increased effectiveness in vivo was confirmed. But the question of cardiac toxicity was unresolved. However, the Laboratory of Toxicology has a contractor testing this point in rabbits, the animal model of human cardiotoxicity. So, arrangements were made to have enough of the adriamycin—DNA complex made to test in rabbits. This is now underway.

Another example is the arrangement described earlier in which Dr. G. A. LePage has agreed to study the 6-selenoguanosine series of agents. Activity with TG correlates with the degree of its incorporation into DNA of the cell. To study this point, labeled drug must be made and its incorporation into the DNA of a series of tumors with varying responsiveness determined. If radioactive Se can be "exchanged" into the agents by the procedure used with S^{35}, Dr. LePage will be able to tell us whether the Se-drugs are the same or different from TG in mechanism of action and a decision on their potential clinical value can be made.

There are many reasons for preparing analogs. The one most frequently used is to obtain a more active agent. This is the aim most easily tested as well. However, in retrospect, its yield of clinically useful agents is not very high. Two other more direct and important reasons for synthesizing analogs are: (1) To achieve the degree of solubility necessary for formulation. The Baker antifol is an example of this; the same in vivo activity was retained while the water solubility was increased from ~ 1 to > 80 mg/ml., and (2) To eliminate the elements which contribute to clinically important toxicities, while retaining the useful anticancer properties. Adriamycin-DNA may become an example of this.

In the study of analogs, establishing the test system to quantitate undesirable toxicities, unlike the testing for increased solubility and antitumor activity, may be long and demanding. So too will be studies to search among agents which are chemical analogs, but which may have either a different mechanism of action (TG and 6MP for example) or proceed along a different biological pathway to reach their target (6MeMPR and 6MP).

Thus the definitive study of each drug analog series presents a different problem and will require different kinds of capabilities for solution.

Knowing the mechanism of action of the parent drug of any analog series is at the heart of study of the series. DRD is currently very limited in its capacity to supplement by contract the relatively few voluntary studies on drugs of interest to DCT. At the very earliest time that money can be obtained, this capability must be materially expanded if progress in the study of analogs is to proceed. It is to be hoped that the coming year will see steps in this direction taken.

MOLECULAR BIOLOGY AND METHODS DEVELOPMENT SECTION

DRUG EVALUATION BRANCH

DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

The Molecular Biology and Methods Development Section (MBMD), DEB was newly formed in August 1973. It is staffed by four senior professionals (including two commissioned officers and one senior staff fellow) and five technical and clerical employees. The principal objective of this section is to conduct basic and developmental research leading to the establishment of new and improved biological and biochemical drug evaluation assays by coordinating both intra-mural and contract supported laboratory programs. Specifically, these objectives are to be achieved by pursuing fundamental aspects of (a) the growth kinetics of tumor cells and normal cells in vivo and in vitro, (b) the effects of potential antitumor drugs on the growth kinetics of tumor cells and normal cells in vivo and in vitro, (c) identification and characterization of drug-receptor site interactions, (d) the development of novel in vivo and in vitro screens of greater predictive value for specific organ directed antitumor specificity, (e) the development of rational bases for the prediction of optimally effective combined modalities of treatment and treatment schedules.

The section has primary or significant responsibility for four contracts, (Univ. of Pennsylvania, 12188, Britton Chance, Principal Investigator, "Screening for Inhibitors of Mitochondrial Activity"; Atomic Energy, 10056, Robert Tobey, Principal Investigator, "Study of Reversibly-Arrested Cell Populations (in vitro) for the Screening of Antineoplastic Agents"; Allegheny General Hospital, 43730, Haggeman & Lesher, Principal Investigators, "Biological Effects of Chemotherapeutic Drugs on In Vivo Cell Populations, Normal and Malignant"; and Litton Bionetics, 33741, Sagar Sethi, Principal Investigator, "Screening of Compounds for Potential Antienzymatic, Antiviral and Antitumor Cell Activity").

The section also has collaborative responsibility for three contracts (Atomic Energy Commission, 43725, Mullaney & Crissman, Principal Investigators, "Development of a Cell Sorting and Analysis System Applicable to Tumor Cell Characterization and Kinetic Studies"; Southern Research Institute, 60029, Lee Bennett, Principal Investigator, "Certain Biochemical Problems Associated with the Current Effort to Advance the Status of Cancer Chemotherapy"; and M. D. Anderson, 61156, Ti Li Loo, Principal Investigator, "Supplement to Pharmacologic Studies of Antitumor Agents: The Evaluation of the Effects of Antitumor Agents at the Cellular Level").

Significant Accomplishments:

Direct Contract Activities

Atomic Energy Commission (10056), Robert Tobey, Principal Investigator. During the past year primary emphasis has been placed upon a detailed comparison of the effects of BCNU (NSC-409962), CCNU (NSC-79037), and MeCCNU (NSC-95441) on the cell cycle progress of synchronized CHO cells. All three slow the rate of DNA synthesis but allow completion of S-phase. CCNU and MeCCNU did not allow cells to progress out of G-2 phase, while BCNU in addition slowed the rate of progress through G-1 phase. At the present time a correlation of these effects with their cell killing activities is being done. In addition, a similar concurrent comparison of Streptozotocin (NSC-85998) and its β-chloroethyl analog (NSC-178248) is being done. A technique was developed that allows a rapid determination of the distribution of cells through the cell cycle in only 20 minutes. This method uses the binding of mithramycin to the DNA of unfixed cells as a measure of cell DNA content and is being adapted to a project by Dr. D. Duell, Immunology Branch, CBD.

The longer range objective of these studies will be to study the "solid tumor active" chemical drugs nitrogen mustard (NSC-762), phenylalanine mustard (NSC-8086), cyclophosphamide (NSC-26271), MTX (NSC-740), fluorouracil (NSC-19893), FUdR (NSC-27640), adriamycin (NSC-123127), and hexamethylmelamine (NSC-13875), with a view toward providing a solid data base for both understanding the clinical activities of drug combinations and suggesting the appropriate selection of drug combinations.

Allegheny General Hospital (43730), Haggeman and Lesher, Principal Investigators. This contract project was initiated in January 1974 but a study of the relative cytocidal activity of BCNU on mouse gastrointestinal crypt cells and on a P815X2 mastocytoma had been completed in this laboratory during the prior year. When LD_{10} doses were used, no significant decrease in crypt number occurred although a period of inhibition of DNA synthesis was produced. A second dose, given at the nadir of DNA synthesis, did not reduce the number of crypts. The cell killing effect on the mastocytoma depended on the site of the tumor, the primary tumor and lymph node metastasis being much more resistant than hepatic and splenic metastasis. It is anticipated that a similar study on adriamycin and bleomycin (NSC-125066) will be provided.

M. D. Anderson, Univ. of Texas (61156). A supplement to a pharmacology project was recently approved with Drs. Drewinko, Humphrey, Rao, and Hsu as the investigators. This group will provide information on the effects of drugs on chromosomal structure, cell culture (including human cell lines) cytocidal properties alone and in combinations, and cell cycle phase and progression sensitivities. The selection of drugs for study will be made by us in collaboration with Drs. Loo and Freireich of M. D. Anderson.

Atomic Energy Commission (43725), Mullaney and Crissman, Principal Investigators. This contract, also very recently approved, will provide the DCT with a flow microfluorometer capable of cell sorting based on cell impedance, light scattering, and fluorescent properties. The use of this instrument for DCT intra-mural and collaborative extra-mural projects will be coordinated by Drs. Shackney and Bono. In addition, Dr. Crissman will pursue the technical aspects of the preparation of solid tumors, human clinical materials and experimental animal tumors (LL carcinoma and B16 melanoma) in a form suitable for use in the flow microfluorometer. The longer range objectives of these activities are to obtain cell kinetic data on human and experimental tumors and to measure drug effects on these systems to direct therapy based on individual tumor response.

Southern Research Institute (60029), Lee Bennett, Principal Investigator. The results of this project are being described in detail in the Biochemistry Section, DEB report. Briefly, biochemical studies on the clinically active agents BCNU, 5-fluorouracil and 6-thioguanine (NSC-752) were conducted and in addition on several drugs being developed for clinical trials: 3-deazauridine (NSC-126849), Inox (NSC-118994), tricyclic nucleoside (NSC-154020), and "Crystalline Antibiotic" (NSC-135758). This is the last year of this contract project. Renewal will be subject to competitative procurement on the basis of RFP-74-42 which was issued February 12, 1974.

Litton Bionetics (33741), Sagar Sethi, Principal Investigator. The contract project has completed its first year under the direction of Dr. T. O'Connor as Project Officer. During this time 40 Rifamycin compounds were tested for (a) in vitro effects on reverse transcriptase (SSV), DNA-Polymerases I and II, RNA-Polymerases I and II, poly A-polymerase and fructose diphosphatase, (b) in vivo effect on DNA, RNA and protein synthesis, cell proliferation and MSV focus formation in 3T3 cells. They were grouped into three clases. Class I, Rifamycins B, O, S, and SV all inhibit viral focus formation but do not selectively inhibit reverse transcriptase. Class 2, several of the cyclo-alkyl hydrazones of 3-formyl-Rifamycin SV (NSC-133100), one of which inhibits focus formation by Feline Sarcoma Virus in human spleen cells but not by MSV in mouse 3T3 cells. Class 3, consists of derivatives of benzyl-demethyl-Rifampicin (NSC-143481) which has significant selectivity in the inhibition of reverse transcriptase. Currently, a similar study on 56 drugs with known clinical or experimental animal tumor activity is being performed. In addition, a series of seven nucleosides with covalent bond forming ability, synthesized by Ash-Stevens under contract with the Carcinogenesis Branch, were screened. One of these, a tetra-thymidine oligomer (NSC-195186) was active as an inhibitor of MSV focus formation in 3T3 cells at a concentration of 4 to 8 nanogram/ml while having little effect on cell proliferation at concentrations as high as 2.5 to 10 μgrams/ml. These results need to be confirmed and extended to the hexa-thymidine oligomer (NSC-211403) which is now available.

University of Pennsylvania (12188), Britton Chance, Principal Investigator. During the past year a series of 13 drugs were evaluated for their effects on electron transport in isolated mitochondria and on respiration in intact Ehrlich ascites cells. Listed in order of decreasing molar activity they are VM-26 (NSC-122819), Ellipticine (NSC-71795), Adriamycin, Me-CCNU, CCNU, Daunomycin, Ethidium Bromide (NSC-522843), Diamine dichloroplatinum (NSC-119875), Picolinaldehyde thiosemicarbazone (NSC-107392), ICRF-159 (NSC-129943), Emetine (NSC-33669), Hydroxyurea (NSC-32065), and Cordycepin. An initial indication that daunomycin and adriamycin were selective for heart mitochondria was not confirmed. However, their significant activity in mitochondria may relate to their myocardial toxicity. Currently, resources are being organized by Dr. Chance to pursue the spectrophotometric analysis of the action of daunomycin and adriamycin on electron transport in intact perfused heart and liver. There is a possibility that the basis of their mycardial toxicity will be defined from these studies and that methods of prophylaxis may be developed.

Collaborative Activities

During the summer of 1973, Dr. Mario Gosalvez of Clinica Puerta de Hierro, Madrid, Spain worked in Dr. Chance's laboratory and after returning to his own laboratories has continued to evaluate program drugs for effects on respiration of isolated mitochondria and intact Ehrlich ascites cells. He has identified five drugs with high activity: Baker's Antifol (NSC-139105), trityl thioalanine (NSC-83265), "Crystalline Antibiotic (NSC-135758), thalicarpine (NSC-68075), and an ellipticine analog (NSC-141527). A series of drugs were classified as inactive: D-tetrandrine (NSC-77037), Asaley (NSC-167780), hexamethyl melamine (NSC-13875), Chromomycin (NSC-58514), gallium nitrate (NSC-15200), amino-thiadiazole (NSC-4728), Inox (NSC-118994), galactitol diepoxide (NSC-132313), and Bleomycin (NSC-125066). He is proceeding to evaluate the active drugs in more detail to determine the possible relationship to therapeutic activity.

In colloborative effort between the intramural laboratory of MBMD and Dr. Bijoy Bhuyan of Upjohn Company (Contract 33707) a study of the effects of agents active in the treatment of human breast cancer was undertaken. The objective is to determine the cycle phase cytocidal and cycle phase progression effects of one agent on the cytocidal and progression effects of a second. So far, 5-fluorouracil, methotrexate, vincristine and adriamycin have been evaluated as single agents as a preliminary to the combination studies. The availability of 4-peroxycytoxan (NSC-176986) and 4-hydroperoxycytoxan (NSC-181815) will allow the *in vitro* evaluation of this "activated" cytoxan alone and in combination in these studies. Under support of this same contract, it is planned to evaluate four preclinical drugs for cell biological and molecular biological activities: Indicine-*N*-oxide (NSC-132319), VP-16 (NSC-141540), Nitidine (NSC-146397), and Homoharringtonine (NSC-141633).

The following drugs were sent to Dr. Samuel Barranco, University of Texas at Galveston, for evaluation of cycle phase cytocidal activity: tritylthioalanine (NSC-83265), Inox (NSC-118994), galactitol diepoxide (NSC-132313), "Crystalline Antibiotic" (NSC-135758) Anguidine (NSC-141537), tetrahydrohomofolate (NSC-139105), Neocarzinostatin (NSC-157365), and Asaley (NSC-167780). Data on these studies is not yet available. Small quantities of carminomycin (NSC-180024), daunomycin, adriamycin, lapachol (NSC-11905), and dichloroallyl lawsone (NSC-126771) were sent to Dr. Karl Folkers, University of Texas at Austin for evaluation as inhibitors of the Coenzyme Q dependent step in mitochondrial electron transport.

In discussion with Dr. David Yesair of Arthur D. Little, Inc. (Contract 33727) plans were made to institute a biochemical screen for the ability of new potentially clinically active agents to bind to the nuclear lipid of L1210 cells which binds vincristine and terephthalanalides. In discussions with Dr. Corwin Hansch, Pomona College (Contract 33703) tentative plans were made for structure-activity study of thymidylate synthetase which might lead to more effective inhibitors.

New Contract Activities

During this year the MBMD evaluated 14 unsolicited proposals for drug related biochemical research and 3 unsolicited proposals for drug related biological research. Most of the unsolicited proposals were relevant but could not be considered without competition. On August 24, 1973 a synopsis was published in C. B. D. for Biochemical and Molecular Biological Evaluation of Antitumor Drugs. In response to this 78 individual resumes and 28 letters of inquiry were received. An Ad Hoc Source Evaluation and Selection committee selected 19 projects to receive an invitation to respond to the RFP. On February 12, 1974 the RFP was mailed to 128 institutions. At this time, 21 proposals have been received.

Workshops and Symposia and Reports

Staff members of MBMD were responsible for the following workshop and symposium activities: (a) Presentation of a lecture on cycle-dependent therapeutic agents at the First Annual Life Sciences Symposium "Mammalian Cells: Probes and Problems" sponsored by the Los Alamos Scientific Laboratory and N.C.I., (b) organizing and participating in an Interdisciplinary Session in Cellular Kinetics and Pharmacokinetics at the 4th Joint Working Conference DCT, NCI. In addition, a workshop on approaches to defining the biological and biochemical basis of Daunomycin and Adriamycin cardiotoxicity has been planned among intramural and contract scientists from MBMD, Toxicology Branch, and Medicine. A session on Electronic Cell Sorting techniques is being organized as part of the N. I. H. Symposium on Recent Developments in Research Methods and Instrumentation to be held in October 1974. Reports on the Biochemical Activities of amino-thiadiazole, galactitol diepoxide, dichloroallyl lawsone, and 3-deazauridine were writen for inclusion in the clinical brochures for these drugs.

Intra-mural Research Projects

A screening protocol to determine the effects of drugs on the cell cycle progression of L1210 cells in vitro was devised and evaluated. It was found that when drugs effects were measured after 6 hours of exposure, the effect on progression as measured by changes in cell DNA content distributions are constant over a range of proliferation rate from 40% to 60% of control. A series of eleven drugs of known clinical or experimental tumor therapeutic activity were screened: 5-fluorouracil, Hydroxyurea (NSC-32065), Methotrexate, Ara-C (NSC-63878), 6-MP (NSC-755), HN_2 (NSC-762), BCNU, Adriamycin Camptothecin (NSC-100880), BUdR (NSC-38297), and AT-125 (NSC-163501). In addition a series of six highly cytotoxic but experimental tumor inactive drugs were also screened: NSC Nos. 145810, 145813, 146854, 147427, 148342, and 148596. The results of this screen are currently being evaluated to see if the progression effects of the active drugs can be distinguished from those of the toxic but inactive ones. In separate studies the effects of Macromomycin (NSC-170105), Cytochalasin B (NSC-107658), Colcemid (NSC-3096), and NSC-145813 (a drug which apparently inhibits karyokinesis) on cell cycle progression were evaluated. In collbaoration with Dr. E. Canellakis of Yale University (Contract 12339) the effects of a newly synthesized spermidine-diacridine on the cycle progression effects of HeLa cells were studied. In collaboration with Dr. Marc Straus (Washington V. A. Hospital) the effects of tumor age on S-180 (in vivo) cycle progression were evaluated. An extensive study of the staining method for measuring cell DNA content was conducted. Factors such as the effects of cell concentration, pH, ionic strength, light, temperature, fixative type, stain type, acid hydrolyses condition, nucleotide base composition of DNA and instrument laser light intensity were evaluated.

Publications:

See current report Drug Evaluation Branch, Office of the Chief.

Serial No. NCI-3579
1. Division of Cancer
   Treatment, DR&D
2. Drug Evaluation Branch
3. Molecular Biology and
   Methods Development Section
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effects of Chemotherapeutic Agents on L1210 Cell Cycle Progression and L1210 Cell Nuclear Proteins.

Previous Serial Number: Same

Principal Investigators: Vincent H. Bono, Jr. and Robert L. Dion

Other Investigators: David Abraham

Cooperating Units: Department of Pharmacology, Yale University, School of Medicine (Dr. E. S. Canellakis) and Medical Oncology, Washington V. A. Hospital (Dr. M. Straus)

Man Years:

Total : 2 1/2
Professional: 1 3/4
Other : 3/4

Project Description:

Objectives: This project is concerned with the development of techniques for the analysis of drug effects on the progress of cells through the proliferative cell cycle and for their application to (a) the characterization of therapeutically active drugs and (b) the possible selection of agents with potential therapeutic activity. A second concern of this project is the development and application of techniques for the analysis of drug effects on cell nuclear proteins and their relationship to cell survival.

Methods Employed:

L1210 cells were grown in suspension cultures and cloning efficiencies determined by growth of colonies in soft agar. Cell DNA content was determined by fluorescent-Feulgen staining and analyzed by a commercially available flow microfluorometer (BioPhysics Systems, Inc.) and multichannel analyzer (Northern Scientific). Cell DNA content distribution data was processed and plotted by computer. Cell proteins were labeled with isotopic valine or tryptophan and

DNA with thymidine. Cell nuclei were isolated by homogenization in a non-ionic detergent and centrifuged through sucrose. Isotopes were measured by standard methods of liquid scintillation counting.

Major Findings:

1. Screening Protocol for Drug Effects on Cell Cycle Progression.

A screening protocl to determine the effects of drugs on the cell cycle progression of L1210 cells (*in vitro*) was devised and an evaluation was begun to see if therapeutically active drugs can be distinguished from highly cytotoxic but therapeutically inactive ones. The method depends upon the fact that the DNA content of a cell can be used to place the cell in one of the several phases of the proliferative cell cycle. In an exponentially growing population the relative frequency of cells having a specified DNA content is determined by the relative time lengths of G1, S and G2. Any factor that influences these relative time lengths will alter the DNA content distribution (DCD) in a characteristic way. Since the proliferation rate is a reflection of the cell cycle progression process it was decided to compare drugs on the basis of the concentration which produces a proliferation rate of 50% of control. In initial studies with fluorouracil, hydroxyurea, BCNU, and HN_2 it was found that the DCD curves obtained after a 24 hour drug exposure were very sensitive to growth rate. Significant changes in the DCD curves occurred over a proliferation rate range of 40% to 60% of control, making it difficult to compare two drugs if their inhibited growth rates are not precisely identical. However, when DCD curves are measured after a 6 hour drug exposure the results for a single drug were constant over a range of proliferation rates from 40% to 60% of control. In order to compare different drugs the following system of analysis was devised. A set of reasonable but arbitrary values were chosen as the basis of assigning a cell to G1 phase, early S phase, late S phase and G2 phase. The fraction of the population in each of the phases was computed and the difference from that of the untreated control was taken. Thus the effect of a drug is described by four values which reflect either an increase or decrease in the population of these four phases. A series of eleven drugs of known clinical or experimental tumor therapeutic activity were screened in this way: fluorouracil, hydroxyurea, methotrexate, cytosine arabinoside, 6-mercaptopurine, nitrogen mustard, BCNU, Adriamycin, Camptothecin, BUdR and AT-125. In addition a series of six highly cytotoxic but experimental tumor inactive drugs were also screened: NSC-145810, 145813, 146854, 147427, 148342, and 148596. The results are currently being analyzed to see if the active drugs can be distinguished from the toxic but inactive ones. One example of a simple pattern assignment is presented. If an increase or decrease in G1 or early S phase of greater than 5% of the total population occurs, then a value of +1 or -1 is assigned, otherwise a value of 0 is assigned. Similarly if a change of 2.5% of the total population occurs in late S phase or G2 phase, values of +1 or -1 are assigned. When the results are analyzed in this manner the following pattern emerges.

NSC Number	Drug	G1	early S	late S	G2
19893	FU	0	+1	0	-1
32065	HU	0	+1	0	-1
755	6-MP	0	+1	0	-1
38297	BUdR	0	+1	0	-1
740	MTX	0	0	0	0
183501	AT-125	0	0	0	-1
145810	Toxic drug	0	0	+1	-1
148342	Toxic drug	0	0	0	0
762	HN_2	-1	+1	+1	+1
100880	Camptothecin	-1	+1	+1	+1
63878	Ara-C	-1	+1	0	+1
123127	Adriamycin	-1	0	+1	+1
409962	BCNU	-1	0	+1	+1
147427	Toxic drug	-1	+1	+1	0
146854	Toxic drug	-1	0	0	+1
148596	Toxic drug	-1	0	0	+1
145813	Toxic drug	-1	-1	+1	+1

This analysis does not lead to a clear distinction between actives and toxic drugs. However, certain patterns for the actives do appear, i.e. if G1 is 0 early S is +1 and G2 is -1, and if G1 is -1, then G2 is +1. Although three of the six toxic drugs have this second pattern their early and late S patterns differ from the actives. An expansion of the data base as well as continued exploration of the analysis is needed before a conclusion can be reached.

2. Evaluation of Cell Cycle Progression Effects of Drugs of Special Interest

Special studies of the cycle progression effects of NSC-170105(Macromomycin) NSC-107658 (Cytochalasin B), NSC-3096 (Colcemid) and NSC-145813 were performed. Macromomycin was found to produce a major block in progression during G2 phase and a slowing of progression through late S phase. At higher concentrations progress through all of S phase was slowed. There was no decrease in progression from G1 into S phase. Cytochalasin B has no effect on cycle progression but does prevent cytoplasmic division (inhibits cytokinesis). This results in binucleate cells, each of which replicate their DNA and again undergo nuclear division, producing tetranucleate cells. It was not determined how far this process will go in L1210 cells but after 24 hours at 5 μg/ml concentration, survival was reduced to 2%. When surviving colonies were allowed to grow out only mononucleate cells were found. Colcemid, a well known inhibitor of cycle progress in metaphase had no effect on the rate of progress of cells out of G1 phase and through S phase. There was an indication that the DNA of metaphase chromosomes does not stain with as great an intensity as that of interphase chromatin.

One of the cytotoxic but therapeutically inactive drugs tested in the screen,NSC-145813, was found to allow endoreduplication of cell DNA. Cells with DNA contents from tetraploid to octaploid were produced. Under fluorescence microscopy these cells were larger than normal and contained one large nucleus. A small percent of the cells contained many small nuclei. Studies are in progress to see if this prevents chromosome condensation or if it interfers with normal karyokinesis.

3. Collaborative Studies

In collaboration with Dr. E. Canellakis of Yale University, the effects of a newly synthesized double DNA-intercalating drug, spermidine-diacridine (SPEDAC) were evaluated. A one hour exposure of HeLa cells to 6 $_\mu$g/ml SPEDAC allows cells to undergo two to three more cell divisions and then proliferation ceases. These cells retain their structural integrity for as long as 12 days. DCD curves of these cells over the time period shows no significant change from control. This indicates that when proliferation ceases cells tend to arrest in all phases of the cycle with equal frequency. After 12 days there was no sign of any degradation or loss of cell DNA, suggesting that they may still retain proliferating potential. Indeed, Dr. Canellakis found that the addition of spermidine to the arrested cells does allow resumption of cell proliferation.

In collaboration with Dr. Marc Straus of Washington,V. A. Hospital the effects of tumor age on S-180 (in vivo) cell cycle progression were evaluated. Over the first four days the cell population increased exponentially. This was followed by a progressive slowing of growth to a plateau by day 8 to 9. The median survival time was 13 days. During the exponential phase of growth the DCD curves showed that S phase is long and G1 phase is short relative to the cycle time. As the population aged and approached a plateau, G1 phase became relatively long and S phase became relatively short. These findings are compatable with the interpretation that as the population ages, G1 phase becomes progressively longer both relative to the cell cycle time and also in absolute value. A limitation to the use of DCD curve analysis of cell kinetics is that it allows only a relative measure of cell phase times and does not allow a measure of absolute times.

4. Evaluation of the Fluorescent-Feulgen Staining Methods

An extensive study of the staining method for measuring cell DNA content was conducted because it was found that the staining intensity per cell increases with increasing cell concentration in the staining procedure. This phenomena has also been observed by Dr. Yataganas of Sloan Kettering (using a BioPhysics flow microfluorometer) but not by Dr. Crissman of Los Alamos Scientific Laboratory (using the Los Alamos instrument). It was found that factors such as pH, ionic strength, room light, temperature, stain type, acid hydrolysis conditions, cell concentration during instrument analysis and instrument laser light intensity did not contribute to this phenomena. Fixation of cells in glutaraldehyde rather than formaldehyde was found to significantly reduce this variation in staining intensity but it was deficient in other ways, i.e. it allowed cell clumping and gave a broader distribution of staining intensities. At the present time the only practical solution to this problem is to maintain a constant cell concentration for all samples during staining.

5. Mechanism of L1210 Cell Death by Hydroxyurea.

Last year we reported that the ability of hydroxyurea to kill S-phase cells depended on their active synthesis of DNA. Plateau phase cells which were arrested in S-phase but not actively synthesizing DNA are resistant to the lethal action of hydroxyurea. We also reported that the extent of inhibition of DNA synthesis appeared not to be responsible for lethality since cells survived the slow addition of hydroxyurea over a 4 hour period resulting finally in a reduction of synthesis rate to 5% of control. By contrast, when DNA synthesis rate is reduced to 5% of control over a 30 minute period cell death is extensive. These studies were extended to an evaluation of the role of the rate of inhibition of DNA synthesis. It was found that a 30 minute exposure to 0.5mM hydroxyurea killed all S-phase cells while a similar exposure to 0.3 mM hydroxyurea killed only 40% of the S-phase cells. However, both concentrations reduced DNA synthesis at exactly the same rate, to 8% by 10 minutes and 4% by 30 minutes. Thus the rate of inhibition of DNA synthesis could not be shown to account for the lethal action of hydroxyurea. In summary, then, we find that active DNA synthesis is required for the lethal action of hydroxyurea but that neither the rate of inhibition nor the maximal extent of inhibition of DNA synthesis can account for its lethal action.

6. Special Testing of Drugs for Effects on L1210 (In Vivo).

Since it was reported by Hill of Southern Research Institute that BCNU is probably detoxified by a denitrosation reaction catalyzed by hepatic microsomes, a test of the effects of SKF-525A (NSC-39690) on the therapeutic activity of BCNU was conducted. Using i.c. L1210 and single i.p. therapy, 50 mg/kg of SKF-525A given 5 hours before 10 mg/kg BCNU produced an insignificant increase in life span (ILS) from 63 to 88. Thus no increase in activity of BCNU was demonstrated. However, BCNU by itself was not as effective in prolonging life span as it was expected.

ICFR-159 (NSC-129943), a poorly soluble active agent has been resolved into its two optically active isomers which are more water soluble, NSC-169779 and NSC-169780. A basis for selecting one of these to replace ICRF-159 is needed. The racemic mixture NSC-129943 has been previously shown to both reduce the acute toxicity of adriamycin and daunomycin and to increase their experimental tumor therapeutic activity. A test to evaluate the relative activities of the two isomers on the acute toxicity and activity of daunomycin is underway. The preliminary results indicate that both isomers reduce the toxicity of daunomycin and increase therapeutic activity. There is also a preliminary indication that the S(+) isomer NSC-169780 may be superior to either the racemic mixture or the R(-) isomer.

Proposed Course and Relevance to the Program

We propose to continue to evaluate the possible application of a cell cycle progression effect screen for the selection of drugs with potential therapeutic activity. Plans are being made to undertake a project to develop a flurescent staining method for measuring DNA synthesis rates. This could lead to the ability to measure simultaneously, on individual cells, both their DNA content and their DNA synthesis rate. Such a two parameter distribution analysis would allow a more complete characterization of a population of cells. The effects of tumor type, tumor age and drug action could be described in terms of DNA synthesis rate dependence as well as the relative lengths of the cell cycle phases.

Publications:

See current report, Drug Evaluation Branch, Office of the Chief.

Serial No. NCI-3583
1. Division of Cancer Treatment, DR&D
2. Drug Evaluation Branch
3. Molecular Biology and Methods Development Section
4. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Modification of Division Patterns and Immunogenicity of Neoplastic Cells by Interaction of their Surfaces with Microenvironmental Macromolecules

Previous Serial Number: Same

Principal Investigator: Muriel Lippman

Co-Principal Investigator: None

Cooperating Units: Southern Research Institute (R. Laster), Arthur D. Little (I. Wodinsky), Naval Biomedical Research Laboratory, University of California (A. Hackett)

Man Years: Total : 1
Professional: 1
Other : 0

Project Description:

Objectives:

1. The project aims at exploiting those changes in cell surface which accompany neoplastic transformation with the object of finding cell division inhibitors which selectively bind to transformed cells. To this end polysaccharides, glycoproteins, proteins, and synthetic polymers are to be obtained and tested for surface binding and cell division inhibition activity on a variety of cell types in culture.

2. In the case of manifest cancer, the immune responses evoked by the malignant cells have failed to effect a complete rejection of the neoplastic cells. Evidence exists that attributes this failure to the weak nature of the cancer cell as an immunogen and to the phenomenon of enhancement frequently observed with sarcomas and carcinoma but not with leukemias. Studies are carried out with the aim of increasing the immunogenicity of the neoplastic cells and preventing some aspects of enhancement. In order to distinguish the direct cytotoxic action vs. action due to increased cell mediated immune response of a

given agent, tumors were transplanted to nude mice and treated with the agent. Since these mice do not have T cells, the responses observed are due to direct cytotoxic action of the drug and/or B cell mediated responses. The humoral responses may be separately monitored. The nude mice will also be used to study the response of human tumors to chemotherapy with macromolecules which bind to the surface alone and in combination with other drugs and modalities.

3. Binding of a particular polymer to a cell surface depends on cell type but also on physiological age of the cell with respect to the cell and culture cycle. Conventional as well as new methods of cell separation are developed in order to investigate adsorption as a function of the cyclic behavior of the cells.

4. New methods are to be developed for rapid, microscale, in vitro tests suitable for screening small quantities of compounds which provide more information than measure of the cytotoxic action of the agent. Of special interest are microculture methods forhuman tumor cell lines.

Methods Employed:

Along with standard methods a substantial part of the project efforts are devoted to the further development of unique methods of cell separation, analysis, and culture which are needed to realize the aims and objectives of the project. In vitro/in vivo correlative systems for the TA3 tumors in culture and in A/Heston BDF_1 and nude mice are being used.

Major Findings:

STUDIES WITH SURFACE BINDING MOLECULES

The antibiotic (Macromomycin NSC-170105) isolated from Streptomyces macromyceticus and characterized as a 15,000 mw protein by Umezawa's group in Japan was the focus of continued studies this year. Last year we reported the MCR did indeed bind to cell surfaces of L1210 and TA3Ha cells in culture and could prevent cell division in non-lethal concentrations. Further study of this macromolecule revealed cytostatic concentration of MCR increased the immunogenicity of cultured TA3Ha and L1210 cells when compared to untreated control cells. Culturing alone increases the immunogenicity of transplantable tumor cells but the effect of MCR is over and above any effect of culturing itself. Where a weak histocompatibility barrier already exists, such increase in immunogenicity as is produced by MCR is sufficient to prevent tumor growth entirely, while in syngeneic hosts the increase in immunogenicity leads to a highly significant increase in survival time.

Analysis of the effect of cytostatic concentration of MCR in culture showed that while cells were generllly arrested in G_2, a large percentage of the population endoreduplicated, forming giant cells with excessive amounts of DNA as revealed by Feulgen stain. The distribution and appearance of the DNA was also abnormal.

Studies of the effect of MCR on cultured human cells were undertaken in collaboration with Dr. A. Hackett (Naval Biomedical Research Laboratory, U. of

California). MCR was shown to prevent division of human rhabdomyosarcoma, and melanoma, but was active against normal fibroblasts from the same patients as well.

The effect of MCR on L1210, P388, B16 and Lewis Lung tumors were investigated. MCR is active against all of these tumor systems. The maximum effect obtained with L1210 was +38% ILS, and for P388 was +68% ILS. The effects against B16 and Lewis Lung tumors were more dramatic (>50% ILS) with cures obtained in some experiments for both these tumors. Injections of MCR at the tumor site proved to be far superior to ip injections for the Lewis Lung tumor.

MCR was brought up to DN-2A and received a high priority rating for formulation and toxicology studies. Purified MCR is being sought from Dr. Umezawa.

The results obtained are reported in the following publications:

Lippman, M. M., Abbott, B. J. Modification of Transplantability and Tumor Growth Following Treatment of L1210 and TA3Ha Cells with Macromomycin (NSC-170105). CCR, Part 1, 57(4), 501-503, 1974.

Lippman, M. M. In Vitro Studies on Macromomycin (NSC-170105): Effects on cultured TA3Ha and L1210 Cells. CCR, Part I, 58(2), March-April, 1974.

Lippman, M. M., Abbott, B. J., Baratta, M. Antitumor Activity of Macromomycin (NSC-170105) Against L1210 and P388 Leukemias and B16 Melanoma.
Submitted for publication.

Lippman, M. M., Laster, R., Venditti, J. Response of Lewis lung Tumor to Intraperitoneal vs. local injection of Macromomycin (NSC-170105).
Submitted for publication

STUDIES ON IMMUNOGENICITY:

In order to distinguish that degree of activity obtained by a surface binding macromolecule which is due to its cytostatic/cytotoxic actions and that part which is due to its modification of immunogenicity, MCR was tested on tumors in nude mice. The results of these studies are not yet complete but show that nude mice are an excellent tool for such analysis. It is anticipated that the specificity of MCR and other drugs for various human tumors will be determined in this system as well.

Significance for the Program:

Macromomycin (NSC-170105) has demonstrated activity against the 4 tumors currently used to predict for good clinical activity. The activity against both B16 and LL tumors is especially noteworthy and suggests that MCR is a good candidate for activity against human solid tumors. Its priority rating for toxicity studies is high. The action of MCR on LL tumor indicates that drug delivery may be of special significance. The mode of action of MCR is unique; it exerts its activity via adsorption to the cell surface. It is cytotoxic and at lower concentrations is cytostatic. At cytostatic concentrations adsorption to the cell surface increased immunogenicity of treated cells. MCR is, therefore, a successful prototype of agents our laboratory has a specific mandate to investigate. The DCT program, and DEB in particular, is commited to finding new active agents, especially those with novel mode of action. High reproducible activity against solid tumors with this unique suface binding macromolecule indicates that this molecular class of agents is a fruitful source of active material and supports the rational of the research program stated in the project objectives outlined in this report.

Proposed Course:

(1) Further study on MCR activity *in vitro* and *in vivo*, alone, and in combination with other drugs and modalities is anticipated. Purified material, when obtained from Dr. Umezawa, will be tested in toxicology. (2) The use of nude mice as a tool for evaluation of cytotoxic vs. immune involvement of MCR and other agents will be further elaborated. (3) Study of TA3Ha and TA3K1 tumor systems in culture and *in vivo* as model systems for measurement of cytotoxic activity, modified immunogenicity, immune enhancement, and immunization will be continued. (4) Investigation of a various surface binding macromolecules (lectins, synthetic and natural polyanions, etc.) to determine specificity or binding to particular malignant cell types will be continued. (5) Other aspects of the program as stated in points 3 and 4 under "Objectives" will be carried out as feasible.

Screening Section

Drug Evaluation Branch

Objectives and Significance to the National Cancer Institute

Principal objectives of the Screening Section are the selection and recommendation of new drugs as candidates for development toward clinical trial against cancer and the investigation of means for increasing the therapeutic usefulness of known antitumor drugs. Objectives are attained by (a) screening a large number of synthetic and natural products *in vivo*, and to a limited degree, *in vitro*; (b) conduct of biological assays for directing fractionation of natural products and isolation of active components; (c) in-depth evaluation of materials which emerge from primary screens with emphasis on the influence of factors such as route and treatment schedule that affect therapeutic efficacy; (d) analysis, evaluation, and presentation of pertinent experimental results to Division of Cancer Treatment Staff; and (e) methodology research leading to development of improved assays for drug evaluation. Program is implemented through establishment and coordination of work conducted by contractual arrangement with qualified institutions and through intramural laboratory studies. Activity against experimental tumors *in vivo* is the principal basis for recommending candidate drugs for development toward clinical trial and for studying optimal conditions for drug usage. *In vitro* activity against mammalian tumor cells or microorganisms provides a means of recognizing biological activity among synthetic agents submitted in insufficient quantity for *in vivo* screening, for following fractionation and isolation of active components of crude natural products with correlative *in vivo* activity, for providing information relative to mechanisms of drug action, and for developing assays for estimating effective drug levels in blood and tissues.

I. Staff Functions

A. General

The Screening Section is currently staffed by five senior professionals and two junior professionals, one on detail from the Office of the Chief, DEB. Principal investigators are: B.J. Abbott (Head); M.C. Baratta (CO); R.I. Geran; N.H. Greenberg, and M.M. Macdonald. Staff is supported by five technical and clerical personnel. Staff serves as Project Officers for five contracts devoted predominantly to presumptive *in vivo* screening; Battelle Memorial Institute (N01-CM-12182), Hazleton Laboratories (N01-CM-23704), Illinois Institute of Technology (N01-CM-81021), Mason Research Institute (N01-CM-12259), and WARF Institute (N01-CM-60494) and two contracts for *in vitro* screening methodology; University of Miami (N01-CM-02225) and University of Wisconsin (N01-CM-33733). Another contract was Catholic Medical Center (N01-CM-23703) for the evaluation of a mammary tumor screening system as a human tumor model. In addition, staff, in collaboration with the Chief, DEB; Chief, DDB; Associate Scientific Director and Chief, DR&D; and Scientific Director for Chemotherapy, monitored in vivo and in vitro screening, secondary drug evaluation and methodology tasks performed under multifaceted contracts with Southern Research Institute (N01-CM-12098), Arthur D. Little, Inc. (N01-CM-33727), Bristol Laboratories (N01-CM-90035), Upjohn Co. (N01-CM-43753), Michigan Dept. of Health (N01-CM-33702), Institute Jules Bordet (N01-CM-22053), Japanese Foundation Cancer Research (N01-CM-22054), Mario Negri Institute (N01-CM-33720), Research Triangle Institute (N01-CM-92019), and Microbiological Associates, Inc. (N01-CM-33728). To assist staff in monitoring the increased data emanating from changes in screening protocols, a Screening Services Contract has been negotiated.

In reviewing biological effects of natural product fractions the Screening Section establishes directions of effort under additional DR&D contracts for natural product fractionation and isolation. Staff monitor the work of contract laboratories as indicated; coordinate total effort with other DR&D and Chemotherapy projects; design assays for selection of new active drugs, investigate usefulness and feasibility of new assays; recommend candidate drugs for clinical trial; and prepare data for publication and for review by the Drug Evaluation Committee, Operating Committee, Experimental Therapeutics Area, Clinical Trials Area, and for Chemotherapy Decision Network review.

B. Specific

In conduct of duties staff (1) review proposals for institution or renewal of contracts, recommend new projects to Chief, DEB and prepare materials for Program and contract reviewing bodies; (2) provide guidance and instruction to contract laboratories, monitor scientific and technical sufficiency, and indicate areas needing modification or clarification through frequent communication and project site visits; (3) establish priorities for testing and instruct laboratories to conduct non-routine experiments of special interest; (4) design experimental protocols for screening and detailed drug evaluation, define parameters of response, establish criteria for "activity"; (5) advise Automated Information Section (AIS), DR&D, of data retrieval needs and collaborate in establishment of procedures; (6) advise Mammalian Genetics and Animal Production Section, DR&D, of animal requirements; (7) organize, review and summarize experimental results; (8) instruct laboratories regarding needed additional data; (9) advise natural product chemists under contract to Drug Development Branch of crude materials worthy of fractionation and isolation; (10) conduct studies related to the biological quality control of experimental clinical formulations; (11) conduct studies leading to solution of drug evaluation related problems arising during toxicological study or early clinical trial of new drugs; (12) design and conduct basic research leading to the development of improved assays for screening. Senior Staff serve on the Drug Evaluation Committee (DEC), chaired by the Chief, DEB, which recommends to Chemotherapy Staff materials which meet criteria for acceptance as clinical candidates. Four of the staff constitute the DEC's Data Review Subcommittee (DRS) which submits to full committee materials meeting minimal criteria for significant and reproducible activity. Staff collectively form the Screening Committee (chaired by the Section Head) which assigns materials that pass Decision Network 2 (DN-2) to contract laboratories for initial route and schedule dependency studies and for additional testing against a limited spectrum of signal rodent tumors; and summarizes data for consideration at the Decision Network 2B and subsequent stages of the linear array. Thus, the Screening Section Staff represent a major part of an integrated program for the evaluation of more useful antitumor chemotherapy and the intensive study of new agents.

II. Significant Accomplishments

Accomplishments are summarized below in accordance with performance of specific tasks related to (A) determination of antitumor activity of new agents, (B) selection of candidate drugs for development toward clinical trial, (C) detailed evaluation of new and old drugs of clinical interest, and (D) methodology, research, and development. The major highlight of the current report period was the implementation of the mini-screen which is discussed in detail in the following section.

A. Determination of Antitumor Activity of New Agents (Primary Screening)

Primary screeing consists of the initial testing of synthetics, plant fermentation, and animal products submitted to DR&D for antitumor testing. Protocols for screening are designed to uncover, from results of initial testing, a modest number of materials which are then subjected to progressively exacting requirements designed to select those with the greatest antitumor potential.

Since recent advances in analytical chemistry have made it possible to determine chemical structure and purity from minute quantities of compound, and since synthetic chemists are our major source of new, unique compounds, it became increasingly apparent that the initial screening must be accomplished with a minimal amount of compound. Dr. Goldin, Associate Chief for Laboratory Research, DR&D, in collaboration with Mr. N. Mantel, Biometry Branch, designed a miniscreen to evaluate compounds submitted in quantities smaller than that required by our routine screen. Two screeners, Microbiological Associates, Inc., N01-CM-33728, and Arthur D. Little, Inc., N01-CM-33727, collaborated in a pilot study of a modified leukemia L1210 system in which local tumor growth as well as life span was measured. The dose range, treatment schedule, and number of mice were determined by the amount of drug available.

A feasibility study was then conducted by the same two screening laboratories under the direction of the Screening Section Staff. Seventy coded samples supplied in 5-150 mg amounts were tested at each laboratory. The data obtained from the two labs were then analyzed by Dr. I. Miller, Arthur D. Little, Inc. The results were encouraging with the miniscreen being capable of distinguishing the seven known active compounds from the known inactives. Another 60 samples were run to provide additional data. From these combined data it was concluded that known active agents could be selected with as low a supply as 20 mg.

By May 1973, a radically new presumptive screening protocol had been designed for all new agents entering the screen and testing was initiated at all screening laboratories. The major changes from previous protocols are the use of: 3 mice per test rather than 6, additional dose levels covering a wider range, a single treatment schedule rather than two, and most important, the adjustment of dosage and protocol in accordance with quantity of drug available. Also this year, a major revision of the entire screening program was studied in an effort to increase screening capacity without a concomitant increase in level of effort. The DCT Advisory Committee approved a plan which by July was translated into an operational protocol. This new screen consists of three levels or stages of screening which must be passed before a compound can be recommended for presentation to the DCT staff as a candidate for DN-2. Stage I, the presumptive screen discussed above, discards the vast majority of compounds with a 3 dose level L1210 test on a Q4D, Days 2 and 6 schedule (or a QD1-9, P388 test for Natural Products). At least minimal activity (125% T/C) must be confirmed in a repeat test for a compound to pass to Stage II. Stage II consists of limited schedule dependency testing designed to exploit test conditions in an effort to increase activity. To pass, a natural product must now demonstrate a higher level of activity (150% T/C) in P388 or it must also be active in L1210. A synthetic must maintain L1210 activity. Stage III is identical for synthetics and natural products, and if the compound possesses a unique chemical structure, consists of testing against secondary tumors (B16 melanoma, Lewis Lung Carcinoma, P388 Leukemia) plus full route and schedule dependency testing. Analogues of compounds already in development are compared with the parent compound.

Although the new screening program is now fully operational, the Screening Section continues to identify problems and make minor protocol changes as the need arises. For example, statistical analysis indicated a necessary change to the evaluation criteria for the B-16 melanoma. Currently, activity yields of the mini-screen are under evaluation.

In addition to the L1210/P388 screen, in vitro (KB) screening continues against all new crude plant and animal products and fractionation products from crude plant extractions which had demonstrated correlative in vivo activity. Selected synthetics are also being tested in cell culture in order to elicit indications of biological activity for selection of candidates for testing in tumor systems under development.

During the calendar year 1973, 34,434 new synthetics, 11,661 new crude plant extractions, 2,550 new crude fermentation products, and 2,689 new crude animal products were submitted for screening. A total of 51,334 compounds were screened during this year of transition.

B. Selection of Candidate Drugs Toward Clinical Trial

A total of 961 new synthetic materials passed the initial stages of screening during the calendar year 1973, compared with only 375 in 1972. This increase of more than 156% is a reflection of the greatly augmented input of materials. Unfortunately, however, only 19% of these confirmed in L1210, the highest confirmation rate being in 9KB (63.5%), while in 1972 the confirmation rate in L1210 was 32%. The DRS reviewed 651 materials, and the DEC 90 (Table I). A total of 10 drugs passed DN2. Table II lists the materials which passed DN2 in calendar year 1973 and the experimental basis on which the selection was made. The relatively small number of compounds presented for DN2 consideration is a result of the time-lag required to acquire more compound to complete necessary testing after identification of the material as an initial active. This time lag has, of course, been extended also by the requirement for Stage III testing which was incorporated into the protocol during this calendar year. Also, another factor is the large number of analogues which, though active, are never submitted to the DCT staff because their activity is inferior to that of a parent compound already in clinical trial.

Examination of the basis for selection for clinical candidates shows that 4 agents met DN2A criteria in L1210. This reflects the general experience with this system. Thus, it is felt that the positive correlation between L1210 activity and clinical efficacy indicates that it should continue as our principal primary screen. However, additional emphasis is now being placed upon the use of assays which give experimental evidence that they might predict drugs having usefulness against slower growing tumors.

C. Detailed Evaluation of New and Old Drugs of Particular Interest in the Program of the Division of Cancer Treatment

The section maintains the Selected Agents (SAC) File comprising comprehensive records of experimental results of the diverse assays for 4,741 drugs.

The Screening Section is largely responsible for the detailed evaluation in rodent tumor systems of drugs which passed DN2, including route and schedule dependency tests, combination chemotherapy, studies of comparative effects against fast and slow-growing tumors, and other appropriate studies to determine the extent to which modifications in drug usage might increase each drug's therapeutic efficacy.

The Section reviews and summarizes such information for consideration of Chemotherapy Staff at Decision Points subsequent to initial acceptance. Plans are under way to initiate a study to determine the relative value of various other experimental murine tumor models for predicting anticancer activity in man. Requests have been initiated for testing vs 10 mouse tumor systems with 54 compounds that have reached clinical trials:

These are catagorized as follows:

Drugs with definite activity against at least two major solid tumors in man (S++)

Drugs with clear activity against solid tumors in man but less than S++ drugs (S+)

Drugs with some activity observed but not clearly established against human solid tumors (S±)

Drugs without activity against human solid tumors (S-)

Drugs not adequately tested against human solid tumors (SO)

Five contract laboratories have been asked to participate in this study and, upon completion, data will be forwarded to A.D. Little, for statistical analysis.

1. Route and Schedule Dependency Studies

During the 1973 calendar year, 26 compounds were examined for schedule dependency effects. The murine tumor systems involved were:

L1210 (21 compounds), P388 (8 compounds), Lewis Lung Carcinoma (2 compounds), B16 melanoma (6 compounds), and Madison 109 tumor (1 compound).

The Screening Committee reviewed 21 compounds and submitted analyzed reports to the Chief, Drug Evaluation Branch. These data were also summarized for staff use and for clinical brochures.

Schedule dependency studies usually involve 3 routes of administration and seven treatment schedules. The prime route is intraperitoneally except for highly insoluble compounds, in which case the oral route of adminiatration takes precedence.

During 1973, we have also initiated a study to determine physiological effects that a fasting versus a fed state may have on the oral administration of drugs to both tumored and normal mice. Toward this end, where quantity permits, drug is being administered orally to parallel groups of fasting and non-fasting animals in schedule dependency studies.

2. Combination Chemotherapy

DR&D has been engaged in the conduct of combination chemotherapy trials in animals for a number of years. As a result of increasing reports of clinical effectiveness of drug combinations in recent years, an intensified interest has been generated in the detailed study of drug combinations in animal models. In response to this rise in interest, the DEB has significantly expanded its combination chemotherapy program both in depth and in volume. With combination chemotherapy experiments now being scheduled at seven different screening laboratories and being initiated by a number of DR&D personnel as well as several screening contractors, it became necessary to coordinate testing and reporting of results. An informal group of DR&D staff (including several Screening Section Staff) began to meet regularly to pool information, and to set priorities. An offline computer file is maintained for combination chemotherapy experimental data, including an index which is updated monthly and distributed to regular users of the system. Plans are being discussed to microfilm screeners' summaries and specialized data which the present system cannot process. To date, 17,184 test records have been computer processed, 9,761 of which have been added to the files during calendar year 1973.

The major emphasis in combination chemotherapy this year has been the systematic combining of pairs of drugs which singly have demonstrated clinical activity against solid tumors. The first such group to be completed were the Breast tumor actives. Eight drugs were paired with each other in a series of experiments, the results of which are currently being summarized for presentation to the DCT Combination Chemotherapy Subcommittee. These experiments were conducted by MAI under contract N01-CM-33728 and the data are reported in their 1973 annual report.

Another major project initiated this year was sequential (in contrast to simultaneous) combination of Adriamycin with 6 other drugs of current clinical interest. In each experiment, one drug is given on Day 1 and the other drug is given at various times thereafter. These experiments were designed to determine which drug should be given first and how much time to allow between treatments. These experiments are being conducted at Arthur D. Little, Inc., #N01-CM-33727, Microbiological Associates, Inc., #N01-CM-33728, IIT Research Institute, #N01-CM-81021 Battelle Memorial, #N01-CM-12182, and Southern Research

Institute, #N01-CM-12098. These data are also to be summarized for presentation to the DCT Combination Chemotherapy Subcommittee. These and other combination chemotherapy studies conducted this year are reported in the 1973 annual progress reports of the participating screeners.

3. Biological Quality Control of Experimental Clinical Formulations

As a support function, we are collaborating with the DEB, Clnical Drug Distribution Section, in comparing the *in vivo* activity of formulated drugs with the activity of the bulk compound to insure that the antitumor activity has not been impaired or lost during the process of formulation. The route, regimen and system which is the basis for selection is generally used for this comparison. In addition, effort is also directed in studying the toxicities of unfamiliar vehicles being considered for use in clinical formulations. Such studies are essential inasmuch as the potential toxicity of a vehicle must be examined carefully, especially when the use of intensive parenteral treatment schedules are contemplated. In the past year, comparisons of experimental and/or clinical formulations with bulk material have been carried out against various tumor systems for 19 drugs in development toward clinical trial. In addition, testing of vehicles for two compounds were examined for toxicity in normal animals in our own (DEB) labs.

4. Further Evaluation of "SAC" and Special Compounds

Periodically, the subcommittee of the Drug Evaluation Committee met to review MC 2B, Deferred, MC 1, and Special Catagory materials when new test data was received. With the current emphasis on *in vivo* efficacy rather than cytotoxicity, it was necessary to establish two new catagories. Materials that displayed sufficient cytotoxicity in the 9KB tumor system to be of program interest and which either were inactive or were QNS with insufficient *in vivo* test data to be reviewed were assigned to MC code 1K. Those materials which were only tested *in vitro* were assigned to MC code 1X. During 1973, 205 materials were assigned to MC 1K and 3 materials were assigned to MC 1X, and 45 materials were assigned to MC catagory 1D (Deferred).

In the procurement of materials for the program, occassionally compounds which were SAC were obtained. A review of the testing of these materials was con- ducted and when the supply permitted, if the testing had not been scheduled in the *in vivo* systems of Program interest or was incomplete, this testing was scheduled and the test data reviewed upon receipt.

During 1973 the Selected Agents List was modified. Previously, this list listed for each NSC number only the tumor system in which efficacy was observed. As materials were reviewed by the Drug Evaluation Subcommittee, the efficacy or lack of efficacy in the tumor systems of Program interest was added to the SAC list.

Materials regarded as possible candidates for early clinical trial as well as materials in early Phase I clinical trial were scheduled for special testing in order to determine (a) the relationship of tumor site with that of route injection e.g. whether drugs can penetrate the so called "blood-brain-barrier". The tumor was implanted intracranially with treatment IP (b) whether a combination of the drug to which a tumor has become resistant with another drug can overcome this resistance, and (c) the mechanisms of drug action. When requested, the Screening Section scheduled the testing of materials in routes and schedules other than those routinely employed. For example, in 1973 it was reported that DMSO (NSC 763) enhanced the efficacy of cytoxan (NSC 26271) when it was given in water ad libitum and cytoxan was given IP. This testing is in progress and to date no definitive conclusion can be arrived at.

As a result of inquiries and renewed interest in "Laetrile", Amygdalin MF (NSC B900540) was scheduled for testing alone and in combination withβ-gluco-sidase (NSC 128056) in Leukemia L1210, P388 lymphocytic leukemia, Walker 256 carcinosarcoma, B16 Melanoma, and Lewis Lung Carcinoma. Although toxicity for the combination was observed in all experiments, no marked objective antitumor response was observed. The Lewis Lung experiments were conducted at Southern Research Institute (N01-CM-12098) and the other testing was conducted at Arthur D. Little, Inc. (N01-CM-33727).

In order to facilitate the testing of contract and noncontract analogues of active materials, protocols were established for the testing of these analogues and one screening laboratory (Microbiological Assoc., Inc., N01-CM-33728) was assigned to conduct this testing. In 1973, approximately 1,000 analogues were tested in these protocols.

D. Methodology (Research and Development)

The purpose of research and development studies is to explore improved ways: of calling out by an efficient procedure the less promising test materials submitted for testing, of selecting drugs with the greatest clinical potential; and of describing the most advantageous use of the promising drugs.

Potential modifications for improving the protocol for the random screen are continually being solicited from the contractors and staff, tried, discussed, evaluated, and when appropriate, incorporated. During 1973 several modifications were incorporated into the protocol, including a reduction of the number of mice in initial tests and a system for establishing priorities for development toward clinical trial based on the survival data from several animal models.

Currently-used animal models are constantly being evaluated both from the stand point of the reproducibility of data and from the stand point of the correlation of drug sensitivity of the models and of human neoplastic diseases. In 1973 statistical analyses were made by Arthur D. Little, Inc. (NO1-CM-33727) to determine the reproducibility of data from the L1210 and the B16 models. A study was initiated to accumulate data in the currently-used animal models for all drugs of particular interest in the DCT program so the response of drugs in these models can be compared with the response of the drugs in human malignant diseases.

Experimental model designs of animal models are constantly being evaluated, modified, and developed in an effort to align experimental models with the pertinent clinical situation. For example, the subcutaneously-implanted B16 melanoma is sensitive to different drugs than is the intraperitoneally-implanted B16 melanoma. Data are being accumulated to correlate drug sensitivity of the two models with the drug sensitivity of human neoplastic diseases.

The Sarcoma 180 line used in the past in our program was implanted subcutaneously as a fragment and judged with a tumor-inhibition parameter. A survival parameter is preferable to a tumor-inhibition parameter for several reasons, including the fact that survival time indicates - in addition to tumor inhibition - the interplay of the roles of metastasis and immune response. The growth characteristics of a subcutaneously-implanted fragment of S180 were such that it was not feasible (because of tumor ulceration) to hold the animals for survival data. An ascitic S180, however, is amenable to survival-data gathering and is therefore the preferable experimental model design for S180 survival studies. The S180 experimental model design used in the past is, therefore, being modified, and the Yale Ascitic Sarcoma 180 line implanted intraperitoneally and judged with a survival parameter is now being used at Microbiological Associates, Inc.

At Southern Research Institute an effort is being made to develop a line of C3H mammary adenocarcinoma for surgical adjuvent trials. There are some breast-cancer patients whose primary tumors are quite large and in whom metastasis is not evident at surgery. Early passage lines of C3H tumors are usually noninvasive, nonmetastasizing, and resemble this clinical situation. On the other hand, there are other breast-cancer patients whose primary tumors are quite small and metastasis is extensive at the time of surgical removal of the primary. A line that more closely resembles this clinical situation is now being developed by isolation of tumors, treatment with chemical carcinogens, transplantation of metastases, and intravenous inoculation. This line (which appears to have a high propensity for growth in the lungs, heart, ovaries, adrenals, and kidney) shows promise of being more useful as the surgical adjuvant model.

Additional studies with murine breast models are conducted by Catholic Medical Center utilizing the CD8F1 spontaneous and first generation transplanted tumor, while in cooperation with the Breast Cancer Task Force, studies are conducted at Mason Research Institute which employ the 13762 mammary adenocarcinoma with the rat host. The major thrust of studies in both systems is combination chemotherapy. Animal models aside from the currently-used experimental test systems are being studied in an effort to find models that select drugs that will be effective against human slow-growing or solid tumors.

During the summer of 1972, Dr. Haggeman of Allegheny General Hospital described, during a seminar he gave at the NCI, a line of mouse P815 Mastocytoma which according to his data appeared to be slow-growing in its first *in vivo* generation. In 1973, the Drug Evaluation Branch investigated in its own laboratory the characteristics of this tumor line after it was established as an *in vivo* transplanted tumor.

Many of the anti cancer agents presently being used clinically are ineffective against the currently-used solid tumor models B16 and Lewis Lung: this is particularly true of DNA binders and mitotic inhibitors. These agents are now being studied at Southern Research Institute (Contract N01-CM-12098) against the mouse Ridgway Osteogenic Sarcoma in the hope that it will be a more sensitive tumor and thus serve as a solid-tumor-model about which information can be provided to clinicians so they will know the most advantageous treatment schedules and drug combinations of anti-cancer agents against a solid tumor.

At Microbiological Associates, Inc., Madison 109 lung carcinoma is being studied. This model is of interest for four reasons: (1) approximately 85% of the cases of human cancer are carcinomas; (2) approximately 20% of human cancer deaths are due to lung cancer; (3) Madison 109 is slow growing - it produces a control median survival time of approximately 35 days, compared to the L1210 control median survival time of approximately 9 days; and (4) Madison 109 in its initial testing seems to select immune enhancing agents such as pyran polymer, poly IC, and live BCG; and it seems to be resistant to immune suppressing drugs with antitumor activity against other mouse tumors such as cytoxan, BCNU, 5FU, and MTX.

At Microbiological Associates, Inc., S-91 Cloudman Melanoma and Carcinoma 1025 in mice were studied to see if they would be satisfactory animal models to use for predicting drugs that should be given clinical trial for solid tumors. These tumors were selected by the DEB for further study on the basis of the fact that available data showed that they were sensitive to many of the drugs ranked high for activity against human solid tumors. MAI concluded from their study that - Carcinoma 1025 would not be a satisfactory animal model because of its slow growth rate and poor growth pattern, i.e., ulceration, necrosis, large numbers of "no-takes", and wide range of survival time of tumored control mice. The investigation of S-91 Cloudman Melanoma is not yet completed.

The study of the potential of the first generation transplant AKR as an experimental model was completed during 1973 at IITRI. The conclusion of the study was that this system correlates quite well with the spontaneous system and thus provides a highly efficient prescreen for spontaneous AKR tumor studies.

At Hazleton Laboratories an intracerebrally-implanted mouse ependymoblastoma was used to test 177 compounds of interest to the DCT Program. Using a survival parameter, the model selected fourteen drugs as highly active. These included: NSC-132313, dianhydrogalactitol; NSC-116785, phenester - which produces limiting toxicity; NSC-77213, procarbazine; four nitrosoureas; NSC-756, 8-azaxanthine; NSC-74437, piperazine; NSC-18429, alanine mustard; NSC-109724, isophosphamide; NSC-34462, uracil mustard; and NSC-45388, DTIC.

At IITRI studies are being initiated using a renal cell carcinoma. At this time both the SC and IR models are being explored.

At the Southern Research Institute efforts are being made to develop a mouse colon tumor for use as an animal model to search for drugs for treating colon tumors in man. Seventy-two colon tumors were induced in four inbred mouse strains by chemical carcinogens; 49 were transplanted; and two have thus far survived initial passage, are in serial transplant and appear to be suitable for chemotherapy studies. One is undifferentiated and highly metastatic, and the other is an adenocarcinoma.

Combined Modalities

The combined modality of gamma-radiation plus a drug has been studied at Arthur D. Little. Of the 15 drugs studied in this way against P388 lymphocytic leukemia implanted intraperitoneally, 13 were more active (i.e. survival time was increased) when the drug was administered along with whole-body gamma-radiation than when the drug was administered alone. The two drugs that showed the greatest synergism with gamma-radiation in this study were: nitrogen mustard and cytoxan. The two drug for which survival time was not increased with gamma-radiaiton were: prednisone, and epipodophyllotoxin.

Similar studies are continuing using intramuscularly-implanted B16 melanoma and using intracerebrally-implanted P388 lymphocytic leukemia.

III. Related Projects

In order for the screening program to function properly, all contract laboratories must employ tumor lines with uniform biological characteristics and host animals of uniform high quality. To achieve this uniformity, the Screening Section maintains frozen tumor banks at specified laboratories and from these, new tumor lines are dispensed to all screening laboratories at specified intervals. In addition, the tumor lines are monitored by the Screening Section and if it appears that the characteristics of a tumor line in use may be changing, new tumor lines are requested and sent to all contract laboratories from the tumor bank.

The need for host animals in good health and in large numbers necessitates close liaison with the Mammalian Genetics and Animal Production Section. Selected screening contract laboratories perform studies related to the diagnosis and control of animal disease and to provide the quality control necessary for maintenance of the quality of the animals utilized in the DR&D Program.

The Screening Section, as the major user of the DR&D data processing system, continues to work closely with AIS by advising and assisting in the planning of modifications and the monitoring and review of data necessary to an ongoing data processing system. In addition, we continue to review for need, all requests for special queries to our data files.

IV. Proposed Course of Projects

The Screening Section will continue the supervision of testing and the evaluation of data for synthetic compounds, plant, animal and fermentation products which are submitted to the DR&D. Emphasis will continue to be placed upon several diverse but related projects of interest to the Division of Cancer Treatment Program of NCI. Utilization of in-house laboratory facilities to provide rapid and accurate evaluation of both test systems and compounds of major program interest will contine. This will involve basic research in areas related to cancer chemotherapy and pilot systems aimed at devising new and improved tumor assays prior to their establishment in contract laboratories. Effort will continue both in the intramural program and in the contract areas to devise, implement, and evaluate more sophisticated systems to uncover drugs of potential clinical usefulness. Development of a broader program for drug evaluation against "slow-growing" tumor systems is underway.

ublications

ee Current Report, Drug Evaluation Branch, Office of The Chief.

Table I

Status of Active Compounds

Calendar Year, 1973

New Actives	961
No. of compounds reviewed by:	
DRS	651
DEC	90
Total number reviewed by DRS and DEC	741
Recommended to Chief, DR&D as candidates for Chemotherapy Staff (DN-2) consideration as potential candidates for clinical trial	4
Passed DN-2	10
Route and schedule dependency studies (experiments) scheduled	38
No. of compounds reviewed by Screening Committee	21

Table II

Compounds That Passed Decision Network Two During 1973

NSC No.	Compound Name	Date of Action	Basis of Action
51143	1H-Pryazolo[2,3-a]imidazole, 2,3-dihydro-	1/15/73	3LE21
154890	Berbinium,5,6,7,8,13,13a-hexadehydro-2,3,10,11-tetramethoxy-8-methyl-, salt with acetic acid monoanhydride with sulfuric acid Coralyne, sulfoacetate	1/15/73	3LE21)Replaced 3PS31)NSC-96349 on the basis of solubility
136020	Androst-4-on-3-one, 17 -hydroxy-7 -methyl-, propionate Testosterone, 7 -methyl-, propionate	1/15/73	Breast Cancer Program Coordinating Branch's Cpd.
73754	Uracil, 5-[(2-chloroethyl)-(2-fluoroethyl)amino]-6-methyl-	3/5/73	Russian Clinical Trial
D124921	Discreet	3/5/73	3LE21
154020	1,4,5,6,8-Pentaazaacenaphthylene, 3-amino-1,5-dihydro-5-methyl-1- -D-ribofuranosyl-	3/5/73	3LE21
165563	Bruceantin	3/5/73	3PS31
167780	L-Leucine, N-[N-acetyl-4-[bis-(2-chloroethyl)amino]-DL-phenylalanyl]-, ethyl ester	3/5/73	Russian Clinical Trial
167781	Benzamide, N-[bis(1-aziridinyl)phosphinyl]-2,5-diiodo-	3/5/73	Russian Clinical Trial
151466E	Triethylamine, 2-[p-(2-chloro-1,2-diphenylvinyl)phenoxy]-, citrate (1:1), (Z)- Clomifene, cis-, citrate	5/4/73	Breast Cancer Program Coordinating Branch's Cpd IND-filing notice 4/25/73 received 5/4/73

Serial No. NCI-3578
1. DCT, DR&D
2. Drug Evaluation Branch
3. Screening Section
4. Blair Building

PHS-NIH
Individual Project Report
July 1, 1973 through November 30, 1073

Project Title: Basic Studies for Drug Evaluation Utilizing Antitumor Screening

Previous Serial Number: Same

Principal Investigator: B. J. Abbott

Co-Principal Investigator: D. Abraham

Cooperating Units: Drug Development Branch (R. Engle, P. Davignon); Molecular Biology and Methods Development Section, (DEB), (M. Lippman)

Man Years: Total : 2

Professional: 1/2

Other : 1 1/2 (includes only 1 full-time person, other staffing with part-time employees)

Project Description and Objectives

The intramural laboratory of the Screening Section conducts specific detailed studies related to *in vivo* antitumor efficacy of materials of interest to the Division of Cancer Treatment as well as the development of new and improved testing designs and tumors.

Methods Employed

The murine tumors utilized in these studies were the transplantable L1210 and P388 leukemias, B16 melanoma, Lewis Lung Carcinoma, and the P815 Mastocytoma. New testing regimens and procedures were used in these systems.

Major Findings

I. Studies related to the design of protocols for screening.

A. Experiments were conducted with materials of known efficacy in the L1210 and P388 tumor systems in order to evaluate the proposed - and later adopted - protocol changes of 1) using 3 mice per dose, doses to be used, and injection schedule in L1210 tumor system, and 2) using 3 mice per dose with treatment days 1-9 in the P388 leukemia.

The experimental data obtained indicated that with these materials, the protocol changes were feasible.

B. L1210 and P388 Leukemias

Several experiments were conducted with NSC-138755 - fluorene, 9-azido-2,4,7-trichloro- and NSC-143414 - Benzyl-dimethyl-rifampicin - in one or both of these systems in cooperation with the Drug Development Branch (R. Engle, P. Davignon). NSC-138755 was tested in a comparison of a suspension vs a solubilized form. NSC-143414 was tested in a comparison of the bulk, proposed vehicle, and NSC-143414 prepared in the proposed vehicle experiment. The data, upon completion of the experiments, was forwarded to the Drug Development Branch.

Studies to determine the efficacy of Macromomycin (NSC 170105) in the L1210 tumor system were conducted in cooperation with M. Lippman (Molecular Biology and Methods Development Section, DEB). Three schedules were used - daily days 1-9, every 4th day (days 1,5,9), and every other day (days 2,4,6,8) - post tumor implantation. No efficacy or toxicity was observed at the doses employed. Higher doses could not be scheduled due to the short supply of Macromomycin.

II. B16 Melanoma and Lewis Lung Carcinoma

Studies in both systems were conducted upon the request of members of the DR&D staff to confirm previously reported efficacies.

III. P815 Mastocytoma

Continued testing of specific materials in this system were scheduled in the established SC implanted tumor with the aim of evaluating the characteristics of this tumor.

IV. Natural Product Toxicity

Several contract screening laboratories notified members of the Screening Section that crude extracts of new plants were more toxic than would be expected and necessitating testing at lower doses than previously employed for materials of this nature. A random selection of 147 crude natural products were prepared as described in the DR&D protocol (Cancer Chemotherapy Reports, 3, #2, Sept. 1972) and screened in the P388 leukemia. The experimental data confirmed that extracts of new plant acquisitions were more toxic than in the past several years. This information was reported to Dr. J. Hartwell (Head, Natural Products Section, Drug Development Branch).

Proposed Course

In November 1973, the Screening Section intramural laboratory was transferred to the Laboratory of Experimental Chemotherapy, Tumor Biology Section.

ANNUAL REPORT

LABORATORY OF EXPERIMENTAL CHEMOTHERAPY

DRUG RESEARCH AND DEVELOPMENT

DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

The laboratory conducts fundamental research in experimental chemotherapy through specific programs in tumor biology, biochemistry and immunochemotherapy. These programs include in tumor biology such areas as the investigation of the molecular basis of drug specificity, individualization of therapy and clinical correlations, biology of cell surface in relation to neoplastic transformation, reversibility and therapy, the fudamental basis and therapy of invasiveness and metastasis, models for multiple combination chemotherapy based on biological and biochemical parameters of drug action, and studies of adjuvant chemotherapy. The program in biochemistry includes studies on the biochemical characterization of the cell cycle, the biochemistry of cell surface, alteration of active sites and biochemistry of adhesiveness, the development of biochemical systems for identification and study of new agents and investigations on the biochemistry of drug action. The immunochemotherapy program includes investigation and development of screening and evaluation systems for immune suppression and immune enhancement, investigation of therapy-related immunologic phenomena and selected immunochemotherapy studies in drug chimerism, adoptive immunity plus chemotherapy, humoral antibody plus chemotherapy, antigenic alteration plus chemotherapy, immunogenetics and chemotherapy and immunochemotherapy of viral-induced tumors.

The Laboratory of Experimental Chemotherapy currently includes the Office of the Chief (A. Goldin) and two sections which were initiated during the past year: Tumor Biology (R. K. Johnson) and Immunochemotherapy (E. Bonmassar). It is planned for the two additional sections to be activated during the coming year: Biochemistry (T. R. Breitman) and Bio-Organic Chemistry (M. Rabinovitz).

Two Individual Project Reports are included this year for the Laboratory of Experimental Chemotherapy:

(a) Tumor Biology and Chemotherapy, Serial No. NCI 7100

(b) Immunochemotherapy Studies with Various Murine Lymphomas, Serial No. NCI 3577

During the first year of operation of the Laboratory of Experimental Chemotherapy, the two activated sections, Tumor Biology and Immunochemotherapy, have made good progress in organization of program, determination of future directions and in findings on special problems.

During the next year with the activation of the two additional sections, Biochemistry and Bio-organic Chemistry, and the renovation of the laboratory facility it will be possible to complete program balance. It is considered that the Laboratory of Experimental Chemotherapy will contribute significantly to the future directions of the total Drug Research and Development program and provide a firm collaborative base for generation of clinically pertinent data.

During the past year an important aspect of the program of the Laboratory of Experimental Chemotherapy has been to design and perform preclinical experimental studies which answer specific questions of relevance to clinical cancer chemotherapy. For this purpose a program has been initiated on the definition in experimental systems of optimal sequence, scheduling and dosage for two-drug combinations of proven or potential usefulness in various human tumor types. The finding of sequence dependent host toxicity for methotrexate plus arabinosylcytosine could be of significance for the use of these drugs in combination regimens for the treatment of leukemias. A study of adriamycin plus other clinically active drugs, combination regimens of potential use in several solid tumors of man, is underway.

Structure-activity studies among antibiotics represent another significant portion of the laboratory program. Many anthracyclines are being studied toward the goal of finding compounds with greater activity or less cardiotoxicity than adriamycin. This program has led to the demonstration that rubidazone, a derivative of daunomycin, has nearly the same increase in experimental antitumor activity over the parent compound as observed with adriamycin. Experimental studies of the cardiotoxicity of this derivative carried out by the Laboratory of Toxicology have not shown evidence of cardiotoxicity to date. However, high doses of rubidazone must be tested before a definite conclusion of reduced cardiotoxicity can be reached. Continued study of anthracycline antibiotics is anticipated. Structure-activity studies among actinomycin antibiotics are geared to finding a derivative with a broader spectrum of activity than actinomycin D. Collaborative structure-activity analyses of Drug Research and Development data will be continued.

Another aspect of the program involves the investigation of the biologic properties of experimental solid tumors in order to develop model systems with predictive value for clinical solid tumors. These studies have included evaluation of growth rate and chemotherapeutic response of intravenously implanted Lewis lung carcinoma and carcinomas induced by implantation of *in vitro* virus-transformed BALB/3T3 cells. Both systems have sufficiently interesting features to warrant consideration as models for drug testing.

There has been considerable research effort focused on antigenic changes induced by the antitumor drug DIC on lymphoma cells *in vivo*. The finding that a BALB/c leukemia induced by Moloney leukemia virus can be antigenically altered after one treatment generation with DIC suggests the possibility of using this new approach in the immunochemotherapy of lymphomas. Considerable emphasis will be placed on further investigation of drug-induced antigenic alteration. Other drugs will be examined for similar properties. The fundamental nature of the antigenic alteration will be investigated and the relationship to the origin of tumor cell resistance and collateral sensitivity examined as well as the application to immunochemotherapy.

A collaborative program has been initiated in an effort to grow human tumors in athymic nude mice. Success in this direction could permit development of a murine model in which in vivo chemotherapeutic studies could be conducted in mice bearing human tumors, paralleling the treatment of the patients. The athymic mice may be suitable for this purpose. They are incapable of mounting strong thymus-dependent allograft reactions, but fully competent to perform B-dependent allograft rejections. The primary task is to obtain a high percentage of success of human tumor growth in the athymic mice. For this purpose emphasis will be placed on the investigation of factors influencing the growth of allogeneic and xenogeneic tumor cells in these mice. Surveillance and collaboration will be developed with other laboratories working in the same area.

Investigations will be conducted on drug-induced immunosuppression and immunostimulation. One model involving measurement of the degree of development and maintenance of a chimeric state permitted by immunosuppressant drugs has been utilized for study of standard antitumor agents. A model for measuring immunostimulation has been successfully applied to measurement of the potency of various BCG preparations.

As part of this program the immunopharmacology of B-dependent Hb allograft reactions will be carefully considered in view of the potential for this part of the immunocompetent system involved in bone marrow rejection. This program would appear to be particularly pertinent since bone marrow transplantation in man is considered to be a subject of primary interest in tumor chemotherapy.

Overall, stress is being placed on immunochemotherapy approaches in immunogenetically defined systems.

Serial No. NCI 7100 (New)
1. Laboratory of Experimental Chemotherapy
2. Tumor Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report

July 1, 1973 through June 30, 1974

Project Title: Tumor Biology and Chemotherapy

Previous Serial Number: Part of NCI-3575

Principal Investigator: Randall K. Johnson

Co-Principal Investigator: Abraham Goldin

Other Investigators: J. David Bessman, Bagrat T. Garibjanian, David P. Houchens, Artemio A. Ovejera

Cooperating Units: S. C. Cohen, Walter Reed Army Hospital; H. L. Gabelnick, Division of Research Services, NIH; S. K. Carter, Cancer Therapy Evaluation, DCT, NCI; J. M. Venditti and Staff, Drug Evaluation Branch, DCT, NCI; I. Kline, S. Vadlamudi, and M. Kende, Microbiological Associates, Inc.; I. Wodinsky, A. D. Little Co.; A. Shefner, Illinois Institute of Technology; B. Meyers, Battelle Columbus; J. Meienhofer, Children's Cancer Research Foundation; B. Zbar, Biology Branch, NCI; J.A.R. Mead, Experimental Therapeutics, DCT, NCI

Man Years

Total:7
Professional: 6
Others:1

Plus animal care support from Mammalian Genetics and Animal Production Section, DR&D,DCT,NCI

Objectives:

To undertake fundamental studies in therapy of experimental tumors; to investigate potential and established clinical cancer chemotherapeutic agents with respect to their effect on malignant growth and selectivity; to determine the relationship between various biological properties of tumors and response to chemotherapy; to study structure-activity relationships among major classes of antineoplastic agents; to develop underlying principles of successful cancer therapy. Specific objectives include: study of drug sensitivity differences and control mechanisms in the cell cycle; study of the origin and basis of invasiveness, metastasis and tumor angiogenesis and the effects of drugs on these phenomena; investigation of means for effective therapy and prevention of emergence of resistant tumor cell populations; study of the mechanism of drug action and mechanism of drug-induced cell death; study of the influence of drugs on hematopoietic and other sensitive host tissues in relation to antitumor effect; development of new and improved biological systems for screening and detailed evaluation of potential antineoplastic agents; determination of underlying principles of effective combination chemotherapy and combined modality treatment.

Methods Employed: Standard laboratory procedures were employed.

Major Findings:

I. Schedule-dependent Therapeutic Synergism of Abrabinosylcytosine (ara-C) plus Methotrexate (MTX). (R. K. Johnson and A. Goldin)

Several recent reports in the literature have suggested that therapeutic efficacy of the combination regimen comprised of ara C and MTX is markedly dependent on the sequence of administration of the drugs. *In vivo* experiments suggested that administration of MTX prior to ara C resulted in loss of therapeutic benefit and this was explained as an antagonism of the effects of ara C by MTX. However, *in vitro* experiments have indicated that MTX sensitized cultured tumor cells to the cytotoxicity of ara C.

Our findings confirm the therapeutic synergism of ara C plus MTX and have clarified the above discrepancy by showing that there is enhanced host toxicity when MTX precedes ara C which accounts for the loss of therapeutic synergism for the drugs in this sequence.

II. Growth Kinetics and Interactions of Multiple Solid Tumors. (A. A. Ovejera, J. D. Bessman, R. K. Johnson, and A. Goldin)

Studies were undertaken to evaluate the influence of a primary solid tumor on the growth rate of its metastases and on the growth of a second transplanted tumor. Simpson-Herren has recently demonstrated that, following excision of an established subcutaneously implanted Lewis lung carcinoma, there is a transient depression of labelling index and lengthening of cell cycle duration in lung metastases.

After two days these parameters of tumor growth rate increase to levels greater than those observed in lung metastases of mice with intact primary tumors. Our studies have shown that, following surgical excision of intramuscularly implanted Lewis lung carcinoma, growth rate of lung metastases as measured by lung weight and enumeration of lung nodules dramatically increases. Studies are underway to determine the control mechanisms involved: whether metastases grow more slowly in the presence of a primary tumor due to the nutritional demands of the large tumor mass or whether there is some humoral or immunological control factor released by the primary tumor.

III. Chemotherapy of Early and Advanced Lewis Lung Carcinoma Implanted Intravenously. (A. A. Ovejera and R. K. Johnson)

Implantation of Lewis lung carcinoma cells intravenously preferentially gives rise to numerous tumor nodules in the lungs of mice. Bioassay data indicate that viable tumor cells are present in other organs but macroscopically evident (and clinically significant) disease is only present in the lungs. This may provide a relevant lung tumor model. Intravenous inoculation of 10^5 viable tumor cells killed animals within a very narrow range of days. In 10 experiments the median survival time of untreated control groups was from 19 to 21.5 days.

We assessed a spectrum of clinically active antitumor agents for activity against i.v. implanted Lewis lung carcinoma. Early disease, treatment beginning on day 1 and advanced disease, treatment beginning on day 14, were used in our evaluation. Generally the response of i.v. Lewis lung carcinoma was similar to response of s.c. or i.m. implanted tumor. Cyclophosphamide and the nitrosoureas were curative in early disease and increased lifespan considerably on treatment of advanced disease. Bleomycin, 5-fluorouracil and nitrogen mustard had significant activity against early disease, but were inactive against advanced tumor. Nitrogen mustard, one of the most active agents against human lung cancer, had not been picked up as active by s.c. or i.m. Lewis lung. Methotrexate, adriamycin, vinca alkaloids and hexamethylmelamine were inactive against i.v. Lewis lung.

Combination chemotherapy of advanced tumor with cyclophosphamide plus BCNU showed this combination to be synergistic.

Intravenously implanted Lewis lung carcinoma appears to be a reproducible and highly quantifiable solid tumor model for evaluation of potential antitumor agents. It is a highly resistant tumor which is adaptable to combination chemotherapy trials and offers certain advantages over the s.c. and i.m. Lewis lung carcinoma screens presently in use.

IV. Permeability of Normal and Preneoplastic Bladder Epithelium to Antitumor Agents. (J. D. Bessman and R. K. Johnson)

Carcinoma of the bladder has been treated with some success by topical chemotherapy, most commonly with instillation of thioTEPA. A number of cases of severe myelosuppression have been noted following this treatment. We sought to define the permeability of bladder epithelium in rats to various antineoplastic agents and to determine what effect the induction of bladder tumors would have on passage of drugs from the bladder to the blood stream. Labelled thioTEPA crosses the bladder epithelium in normal rats at a rate proportional to the concentration of drug in the instillate. Transport of thioTEPA does not appear to be saturable at high concentrations suggesting simple diffusion. Induction of bladder carcinoma by dietary FANFT markedly increased the permeability to thioTEPA even at stages when only slight urothelial hyperplasia was the only histologically evident change. Ara C was totally excluded from crossing the bladder epithelium both in normal rats and in rats on the carcinogenic diet. The studies suggest that drugs other than thioTEPA may be better for topical chemotherapy of bladder carcinoma in order to minimize systemic toxicity.

V. Antitumor Activity of Inhibitors of Protein Biosynthesis.
(R. K. Johnson)

Practically all of the clinically useful antitumor agents either inhibit DNA biosynthesis or interfere with DNA function in some way. Most of these agents are myelosuppressive by virtue of these effects. Inhibitors of protein biosynthesis in mammalian cells also have shown evidence of significant antitumor activity in experimental systems and a few of these agents have been in clinical trial with some evidence of therapeutic activity, notably L-asparaginase and emetine. These drugs and other antitumor protein synthesis inhibitors, such as anguidine, are not myelosuppressive. We have initiated a systematic comparative evaluation of antitumor activity of known eukaryotic protein biosynthesis inhibitors. Of the agents tested, emetine and related drugs were the most active. Many of the agents, including some with minimal activity when used alone, were synergistic with BCNU in combination therapy of L1210 leukemia.

Experiments will be performed to determine the relationship of cytotoxicity to the extent and duration of inhibition of protein biosynthesis in order to better understand the potential of this class of drugs as antineoplastic agents.

VI. Effects of Antitumor Agents on Wound Healing in Mice. (S. C. Cohen, H. L. Gabelnick and R. K. Johnson)

The recent heightened interest in the use of chemotherapy as an adjuvant to surgery following resection of primary solid tumors prompted us to evaluate the effects of some of the more active solid tumor chemotherapeutic agents on the healing of surgical wounds. By the use of an Instron tension apparatus the physical strength of skin incisions in mice was monitored 3, 7, and 21 days post-surgery. A spectrum of agents effective in solid tumors and, thus, potentially useful in surgical adjuvant regimens were evaluated for effects on the course of wound healing when administered at a single dose close to the LD_{10} level on the day of surgery. Drugs studied include methotrexate, bleomycin, vincristine, BCNU, 5-fluorouracil, actinomycin D, cyclophosphamide and adriamycin. The latter two agents, being the most effective and broad spectrum solid tumor chemotherapeutic agents, were evaluated in some detail with respect to dose response analysis, influence of schedule and time of administration in relation to time of surgery.

Our findings indicate that wound tensile strength 3 days following surgery was unaffected by drug regimens whereas 7-day and 21-day tensile strength values could be significantly depressed by a single dose on the day of surgery of many of the effective agents. Inhibition of wound healing was related to dose of both cyclophosphamide and adriamycin. The latter agent appeared to impair the wound healing process less than cyclophosphamide and would be a good candidate for surgical adjuvant trials in this respect.

VII. Application of the Stathmokinetic Effect of the Vinca Alkaloids in Developing Synergistic Drug Combinations. (J. D. Bessman, S. Vadlamudi, R. K. Johnson, and A. Goldin)

Published results from this laboratory have shown that partial synchronization of tumor cells in vivo by Vinca alkaloids or colcemid can be applied in therapeutic trials to yield synergistic combinations with cell cycle phase specific antitumor agents. Our previous work with mitotic inhibitors in combination with S-phase specific agents has been expanded to the study of the metaphase arresting agents plus adriamycin, 5-azacytidine and ellipticine, highly active antitumor agents with divergent cell kill kinetic characteristics.

Ellipticine has been reported to be most cytotoxic to cells during the mitotic phase of the cell cycle. Combination treatment of mice bearing L1210 leukemia with vincristine plus ellipticine was inferior to the treatment with ellipticine alone even when ellipticine was administered several hours after vincristine when a proportion of the cell population is synchronized in the mitotic phase. Studies are continuing to determine why in this case cell cycle kinetic characteristics in vitro failed to predict for therapeutic synergism in vivo. Initial results suggest that ellipticine and vincristine have more than additive toxicity in normal animals. The resultant loss of selective toxicity could explain why therapeutic synergism was not observed.

Adriamycin and 5-azacytidine do not show any marked preferential cytotoxicity for cells in a particular phase of the cell cycle. In combination with Vinca alkaloids these drugs were therapeutically synergistic. There was no relationship between therapeutic synergism and the sequence or interval of administration for vincristine plus adriamycin or vinblastine plus 5-azacytidine. These results suggested that the partial synchronization of the tumor cells by the Vinca alkaloids was not a basis for synergism with these drug combinations.

VIII. Detailed Comparison of 5-Fluorouracil and Ftorafur. (B. T. Garibjanian, R. K. Johnson, I. Kline, S. Vadlamudi, D. P. Houchens, and A. Goldin)

Ftorafur is an analog of 5-fluorouracil which is in clinical trial in the U.S.S.R., Japan and in this country. It has been suggested that ftorafur is a depot form of 5-fluorouracil. We have compared these analogues with respect to antitumor activity, toxicity, immunosuppression, resistance and cross-resistance.

In several experimental tumor systems 5-fluorouracil proved to be somewhat more effective than ftorafur. P388 leukemia, which is quite sensitive to 5-fluorouracil, was naturally resistant to ftorafur. Antitumor activity was not dependent on schedule of administration with either agent. An L1210 subline was developed which was resistant to ftorafur and proved to be cross-resistant to 5-fluorouracil. A 5-fluorouracil resistant line of L1210 was also cross-resistant to ftorafur. However, the mechanism of resistance of the two sublines appeared to be different as the ftorafur-resistant line sensitized the host to the toxic effects of both of the analogues, a phenomenon not observed with the 5-fluorouracil resistant subline.

Lethal toxicity of the two agents in mice was quite different. On any schedule ftorafur was considerably less potent than 5-fluorouracil. The toxicity of 5-fluorouracil was more cumulative than for ftorafur and recovery of the host from ftorafur toxicity was more rapid than from 5-fluorouracil toxicity.

There were also differences in the immunosuppressive properties of the analogs. These results are discussed in the report of the Immunochemotherapy Section, Laboratory of Experimental Chemotherapy.

Generally, our experiments indicated that ftorafur may not be acting solely as a depot form of 5-fluorouracil as previously suggested. Although we observed no indication of therapeutic superiority of the Soviet analog its reduced toxicity, particularly on chronic administration, may lead to successful clinical application.

IX. Disease-Oriented Approach to Experimental Combination Chemotherapy. (R. K. Johnson, I. Kline, J. M. Venditti, A. Goldin, and S. K. Carter)

In order to more closely orient the experimental combination chemotherapy program of Drug Research and Development to clinical needs, a program of prospectively testing two-drug combinations of those drugs most active as single agents in given human tumor types was devised. Lists of the most active drugs in the various tumor types were compiled by Cancer Therapy Evaluation, DCT. Appropriate predictive tumor models were selected for testing of two drug combination. The first study was made of those drugs which are active in breast cancer through contract resources at Microbiological Associates, Inc. The following two-drug combinations were found to be synergistic in L1210 leukemia: adriamycin + thioTEPA, adriamycin + melphalan, adriamycin + Vinca alkaloids, cyclophosphamide + thioTEPA, cyclophosphamide + melphalan, 5-fluorouracil + melphalan, methotrexate + melphalan, vincristine + melphalan, thioTEPA + melphalan. In addition, the hormone-sensitive P1798 lymphosarcoma was utilized to study prednisone combinations and showed that 5-fluorouracil + prednisone was synergistic.

Other groups of drugs, such as those active in lung or ovarian cancer, will be tested in combination. Those two-drug combinations which show marked superiority over the single agents when used alone will be studied in more detail to define optimal conditions for use in combination regimens.

X. Detailed Evaluation of Adriamycin Combination Chemotherapy: A Drug-Oriented Approach to Experimental Combination Chemotherapy.

(R. K. Johnson, J. M. Venditti, R. Geran, I. Wodinsky, A. Shefner, B. Meyers, A. Goldin, and S. K. Carter)

Adriamycin, which is one of the most effective clinical antineoplastic agents known, is being widely used in combination regimens for the treatment of a spectrum of solid tumors. In response to the DCT Subcommittee on Combination Chemotherapy this laboratory, in collaboration with the Drug Evaluation Branch, and DCT contractors undertook a detailed evaluation of two-drug combinations including adriamycin and each of the following drugs: cyclophosphamide, methyl-CCNU, melphalan, methotrexate, vincristine and 5-fluorouracil. The purpose of the study was to determine the therapeutic potential of each of the combinations and to find out whether the sequence of administration or interval between doses of adriamycin and the other drugs had any influence on the therapeutic activity of the combinations.

In the L1210 leukemia system it was found that several of the combinations were synergistic, particularly adriamycin plus cyclophosphamide and adriamycin plus melphalan. For adriamycin and cyclophosphamide therapeutic synergism was observed when adriamycin preceded cyclophosphamide but not in the reverse sequence and the best results were obtained when adriamycin was given on day 1 and cyclophosphamide was given on day 5. This dependence on sequence may be related to the lack of activity of adriamycin against advanced L1210 rather than a general property of the combination. This is being confirmed by using advanced P388 which is sensitive to both adriamycin and cyclophosphamide. For adriamycin plus melphalan therapeutic synergism was observed over wide dosage ranges and was not dependent on sequence of drug administration. Therapeutic synergism was observed regardless of the interval between drugs, from simultaneous administration to 5 days between doses.

Experiments will continue until definitive statements can be made about the optimal ways of utilizing adriamycin in combination with these drugs and other effective antitumor agents.

XI. Effects of Adriamycin on Nucleic Acid Biosynthesis by L1210 and P388 Leukemia Cells In Vivo. (R. K. Johnson, B. T. Garibjanian, and A. Goldin)

L1210 leukemia is only moderately sensitive to adriamycin in vivo; optimal courses of treatment result in a definite but not extensive increase in lifespan of tumor-bearing mice. On the other hand, P388 leukemia is very sensitive to adriamycin with extensive increases in survival time and long-term survivors following treatment in the early stages of the disease. In order to determine the biochemical basis of this differential sensitivity we studied the time course of inhibition of DNA and RNA synthesis in these two tumor lines following a single dose of adriamycin to tumor-bearing mice. In the more sensitive P388 leukemia, DNA synthesis in vivo was depressed for an extended time period following adriamycin administration while RNA synthesis returned to control levels after several hours of moderate inhibition. In L1210 both DNA and RNA synthesis were profoundly inhibited for several days. The inhibition in P388 was associated with a disappearance of tumor cells from the peritoneal cavity whereas tumor cell kill of L1210 was not as extensive. It is possible that the prolonged inhibition of RNA synthesis by adriamycin in L1210 cells may interfere with the cytotoxic action of the drug in this cell line.

XII. Use of the Coulter Channelyzer for Monitoring Erythrocyte Dynamics in Patients. (J. D. Bessman)

The Channelyzer attachment to the Coulter-S counter allows a breakdown of the counted particles by size. We have used this instrument to analyze the population of erythrocytes in normal subjects and subjects with various diseases. We have identified changes in erythrocyte size distribution in patients with iron deficiency anemia, pernicious anemia, and following transfusion therapy. We expect to be able to follow changes in size distribution sequentially after transfusion and various antimetabolite therapies, as well as in other diseases.

III. Studies on the Interaction Between O-2,2'-Anhydroarabinosylcytosine (NSC 145668) and Arabinosylcytosine (NSC 63878). (R. K. Johnson, J. A. R. Mead, and A. Goldin)

It has been suggested that anhydro ara-C, which exists in the imino-form, may inhibit deoxycytidine deaminase, the enzyme responsible for rapid inactivation of ara-C. This possibility is considered because, unlike ara-C itself, the ara-C released by hydrolysis of anhydro ara-C is excreted intact rather than being almost completely converted to arabinosyluracil. It was felt that such inhibitory activity of anhydro ara-C might be useful in combination with ara-C to give more prolonged blood levels of the active product. However, in treatment of advanced L1210 leukemia ara-C plus anhydro ara-C were no more effective than anhydro ara-C alone.

Studies with isotopically labelled drugs will be performed to determine whether anhydro ara-C has any effect on the pharmacokinetics of ara-C.

XIV. Structure-Activity Studies Among the Actinomycins.

(R. K. Johnson, J. Meienhofer, and A. Goldin)

The great therapeutic advantage gained in the minor modification of daunomycin to adriamycin has prompted renewed interest in structural modification of actinomycin D to lead, hopefully, to a derivative with a wider spectrum or greater degree of activity. Actinomycins C_2, C_3 and X_2 have minor modifications in the cyclic pentapeptide chains of the actinomycin structure. These three agents were evaluated in comparison to actinomycin D in L1210 and P388 leukemias, B16 melanoma and Lewis lung carcinoma. None of these derivatives showed a significant advantage over the parent compound in this spectrum of murine tumors. Further actinomycin analogues will be acquired by the Drug Development Branch, DR&D and will be similarly evaluated in comparison with actinomycin D.

The lack of advantage of actinomycin X_2 over the other derivatives in *in vivo* antitumor evaluation indicates that caution should be observed in using *in vitro* and biochemical assays as primary guides in analogue development. Actinomycin X_2 was considerably more cytotoxic and effective in binding to DNA than was the parent compound. In *in vivo* experiments this was reflected in a considerable increase in potency but a loss in selectivity as optimal therapeutic effects of actinomycin X_2 were inferior to actinomycins D, C_2 or C_3.

XV. Evaluation of Immunotherapy with Non-Specific Immunostimulators in Lewis Lung Carcinoma.

(R. K. Johnson, D. P. Houchens, B. Zbar, and A. Goldin)

In order to develop an antitumor assay in a solid tumor system for non-specific immunostimulators, the effects of BCG and levamisole on Lewis lung carcinoma were assessed. BCG pretreatment, rather than rendering mice more immune to an implant of Lewis lung tumor inoculated intravenously, made mice more sensitive to low tumor inocula. "Antitumor" activity was observed only when BCG and Lewis lung cells were admixed prior to tumor inoculation. Intradermal inoculation of the admixture failed to produce tumors but the mice were still sensitive to subsequent implantation of Lewis lung tumor.

One published report indicated that levamisole was highly active as primary treatment against early and advanced Lewis lung carcinoma. We, as well as several other laboratories, were unable to reproduce these results, and demonstrated no activity whatsoever for this immunostimulator.

XVI. Chemotherapeutic Model with the K-B-N-Cl-6 Non-producer Clone of In Vitro MSV-Transformed BALB/3T3 Cells.
(M. Kende, R. K. Johnson, and A. Goldin)

Further evaluation of the spectrum of chemotherapeutic response of this interesting solid tumor model system has been carried out. The tumor is highly responsive to alkylating agents including chlorambucil and busulfan which have minimal activity in screening systems now in use. Among the antimetabolites 6-mercaptopurine was highly active, 5-fluorouracil and 5-azycytidine were active, while methotrexate and 5-bromodeoxyuridine were inactive. Of the antibiotics adriamycin was quite active while bleomycin and actinomycin D were inactive. Vincristine and vinblastine were also inactive. The nitrosoureas, BCNU and CCNU, had considerable activity.

It is felt that this solid tumor model may be useful in structure-activity studies among anthracycline antibiotics as adriamycin had definite activity but was not curative.

Significance to Biomedical Research and the Program of the Institute:

As outlined in the Objectives, this project is designed to develop fundamental information on the principles of effective chemotherapy. Studies are initiated in response to current clinical needs. In addition, fundamental studies on animal tumor models are directly geared to the goals of the Drug Research and Development program of the DCT for improved screening and detailed evaluation of potential antineoplastic agents and combination regimens.

Proposed Course of Project:

I. As this Section was formed during the past year, most of the described projects are not as yet complete and work in these areas will continue. Various aspects of the project will be modified in accordance with laboratory findings and clinical interest.

II. Further emphasis will be placed upon defining optimal drug scheduling in combination chemotherapy regimens of importance in clinical medicine.

III. As facilities become available work in cell culture will be initiated to study the mode of drug action and mechanisms of cytotoxicity.

IV. More emphasis will be placed on the study of drug resistant tumor populations with a major aim of establishment and evaluation of tumor sublines resistant to anthracyclines for use in structure-activity studies and investigation of mode of action.

V. Studies of drug action on metastasis and tumor angiogenesis will be initiated.

VI. Structure-activity relationships among antibiotics will be continued, particularly among anthracyclines and actinomycins.

Publications:

1. Goldin, A., and Johnson, R. K.: Evaluation of actinomycins in experimental systems. Cancer Chemother. Rep. 58: 63-77, 1974.

2. Ikawa, Y., Brescia, T. Jr., Johnson, R. K., and Goldin, A.: Chemotherapeutic response of epithelial tumors produced by primary implantation of a non-producer line of murine sarcoma virus-transformed BALB/3T3 cells. Cancer Chemother. Rep. 57: 149-160, 1973.

Serial No. NCI 3577
1. Laboratory of Experimental Chemotherapy
2. Immunochemotherapy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report

July 1, 1973 through June 30, 1974

Project Title: Immunochemotherapeutic Studies with Various Murine Lymphomas

Previous Serial Number: NCI 3577; also part of NCI 3575

Principal Investigator: Enzo Bonmassar

Co-Principal Investigators: David Houchens and Abraham Goldin

Other Investigators: Randall K. Johnson, Bagrat Garibjanian, Artemio Ovejera, Marilyn Gaston and Anna Bonmassar

Cooperating Units: D. Young, Laboratory of Toxicology, NCI; L. Albright and J. Madigan, Surgical Neurology Branch, NINDS; M. Kende, S. Vadlamudi, M. Padarathsingh, Microbiological Associates, Inc., Bethesda, Maryland; M. Fioretti, A. Iorio, Institute of Pharmacology, School of Medicine, University of Perugia, Perugia, Italy.

Man Years

Total: 4
Professional: 3
Other: 1
Plus animal care support from Mammalian Genetics and Animal Production Section, DR&D, DCT, NCI

Project Description:

Objectives:

I. To monitor the growth of neoplastic cells in tumor bearing mice in order to determine and improve the efficacy of chemotherapy, immunotherapy or combined treatments.

II. To set up appropriate models to investigate the interactions between antitumor agents and the host's immune response and the possible application of immuno-stimulating drugs.

III. To study the antigenic changes occurring in normal and tumor tissues following treatment with antineoplastic agents *in vivo*.

Methods Employed: Standard laboratory procedures were employed.

Major Findings:

I. Monitoring of Tumor Cell Growth in Mice Bearing Lymphomas or Carcinomas.

A technique was developed to monitor DNA synthesis associated with tumor cell growth in various mouse tissues *in vivo* and *in vitro*. The method is based on the evaluation of 5-iodo-2'-deoxyuridine-^{125}I (^{125}IUdR) uptake *in vivo* by spleen, liver, lung and other organs, or *in vitro* in peripheral blood. A direct relationship between tumor growth and ^{125}IUdR uptake was found in spleen and blood of B10.A and C57Bl/10 mice bearing compatible radiation-induced lymphomas, or of AKR mice with spontaneous leukemia. Similarly a positive relationship was found in spleen and lungs of C57Bl/6 Cr mice carrying Lewis lung carcinoma. Tumor growth and regression in non-presensitized incompatible recipients or tumor inhibition in presensitized allogeneic hosts were paralleled by ^{125}IUdR uptake values in spleen and peripheral blood. Inhibition of tumor cell proliferation by antineoplastic agents was also reflected in inhibition of ^{125}IUdR uptake in the same tissues.

When an ara-C resistant subline of L1210 leukemia was used, ara-C treatment was not followed by changes in ^{125}IUdR uptake values in the peripheral blood *in vitro* and no increase in survival time was found in treated mice as compared with untreated controls. In preliminary experiments high ^{125}IUdR uptake values were observed in the peripheral blood of patients with acute leukemia.

II. Effect of Antineoplastic Agents on Immune Responses of Mice and Exploitability of Immunostimulant Drugs.

A. Activity of Dimethyl-triazeno-imidazole-carboxamide (DIC) on Mouse Allograft Reactions. (E. Bonmassar, D. Houchens, M. Gaston)

(1) Studies on the duration of effect: at various time intervals

after a single injection of graded doses of DIC, CDF_1 mice ($\underline{H-2}^d/\underline{H-2}^d$) were challenged intravenously with 10^7 cells of L5MF-22 lymphoma of B10.129(5M) ($\underline{H-2}^b$) origin. Tumor take and leukemic death occurred in DIC-treated mice when drug treatment was performed 3-20 days before challenge. These findings indicated that the duration of DIC-induced suppression of allograft reactions is unusually long lasting, as compared with that induced by a classical immunosuppressant drug such as cyclophosphamide.

(2) Attempts to antagonize the immunosuppressant activity of DIC in mice: CDF_1 mice were pretreated with DIC as a single or a multiple treatment schedule followed by injection of 10^7 cells of allogeneic lymphoma L5MF-22 i.v. Various groups of mice were treated after tumor challenge with syngeneic spleen or bone marrow cells, either normal or sensitized against L5MF-22 alloantigens. Additional groups of mice were preimmunized with the same allogeneic lymphoma before DIC treatment. The experiments showed that a subsequent transfer of 10^7-10^8 non-sensitized immunocompetent cells cannot restore an efficient allograft reactivity in mice pretreated with DIC, since all animals died with generalized lymphoma. On the other hand, transfer to the host of sensitized spleen cells exerted protective activity although a certain percentage of the mice succumbed with evidence of leukemic growth. Complete protection was obtained in mice preimmunized with L5MF-22, indicating that DIC treatment was not capable of suppressing the already established immune status.

B. Suppressant Activity of Cyclophosphamide and of an Immunodepressive Virus (IDV) Associated with Lymphoma Cells, in Antilymphoma Allograft Reaction in Mice. (E. Bonmassar, D. Houchens, and A. Iorio)

CDF_1 mice were pretreated with various doses of cyclophosphamide or a series of ultrafiltrates obtained from a number of frozen and thawed transplantable tumor lines. The mice were then challenged with 10^7 cells of L5MF-22 allogeneic lymphoma and on day 8 after challenge the ^{125}IUdR uptake of peripheral blood was determined *in vitro*. High IUdR uptake values were found in mice pretreated with cyclophosphamide or with ultrafiltrates of IDV-positive tumor lines indicating that inhibition of the allograft reaction occurred in pretreated mice. This was also evidenced by the presence of large numbers of lymphoma cells in the peripheral blood of the animals.

C. Suppressant Activity of Antineoplastic Drugs and IDV on Allograft Reactions of Lethally Irradiated Mice Against *Hh* (Hemopoietic-histocompatibility) Incompatible Bone Marrow. (E. Bonmassar, D. Houchens, M. Gaston)

Comparative studies were performed of cyclophosphamide and

and isophosphamide to test their suppressant activity on the allograft reaction of irradiated mice against Hh incompatible bone marrow. C57Bl/6 Cr mice were pretreated with graded doses of cyclophosphamide or isophosphamide. Eight days later the animals were subjected to lethal whole-body irradiation (700 R) and transplanted with syngeneic, or allogeneic Hh incompatible bone marrow from BALB/c Cr donors. Incompatible bone marrow (BALB/c Cr)was rejected by irradiated recipients, whereas mice pretreated with cyclophosphamide accepted bone marrow graft. Comparable doses of isophosphamide seemed to be much less effective in preventing the allograft reaction, indicating a difference of activity between the two drugs on the immune system involved in anti-Hh allograft reactions. Similar experiments were performed in irradiated B10.A(5R) (H-2^i) mice pretreated with IDV and transplanted with Hh incompatible bone marrow from C57Bl/10 Sc Sn (H-2^b) donors. The data indicated that IDV did not exert any depressive activity on the Hh allograft reaction in mice.

D. Immunosuppression and Immunochemotherapy Studies with 5-fluorouracil (5-FU) and Ftorafur (FT). (B. Garibjanian, D. Houchens, R. Johnson, S. Vadlamudi, M. Padarathsingh, M. Gaston)

Studies were conducted in conjunction with Dr. Bagrat Garibjanian of the U.S.S.R. (see Tumor Biology Section report) on the immunochemotherapeutic effects of 5-fluorouracil (and ftorafur).

(1) Immunosuppression

(a) Effects on humoral responses against sheep red blood cells (SRBC). The effects of 5-FU and FT on antibody production to sheep erythrocytes (SRBC) was determined. When doses of the drugs were administered on a single treatment schedule either 2 days before or after SRBC, immunosuppression was observed with both drugs. Greater suppression was seen when the drugs were given after the SRBC but there was no significant difference in the degree of suppression conferred by the two drugs. However, the results of the effects of 5 daily injections of the 5-FU and FT on SRBC titers indicate that 5-FU is more immunosuppressive than ftorafur at equitoxic doses.

(b) Effects on colony forming capacity of spleen and bone marrow cells and antilymphoma allograft reactions. The effect of various doses of a single treatment with 5-FU or FT on spleen and bone marrow cells and their ability to form colonies was tested. 5-FU had a slightly greater inhibitory effect on spleen cells and colony forming units than FT. Both drugs showed marked effect on colony forming units of bone marrow cells.

To further test the suppressive effects of the drugs, an FT resistant L1210 tumor line was inoculated into allogeneic animals which received the drugs on a 5-day schedule from one day before tumor inoculation to 3 days after. The 5-FU was highly suppressive at all doses while the FT was only slightly suppressive at equitoxic doses.

(2) Immunochemotherapy

The response of 6C3HED tumor to various doses of 5-FU or FT plus immunotherapy (BCG or Levamisole or OK-432 or Poly I:C) was assessed. Some combinations seemed beneficial. These were 5-FU plus BCG or Levamisole and FT plus Poly I:C.

E. Nude Mice Studies. (D. Houchens, E. Bonmassar, P. Carbone, A. Ovejera, R. Johnson, M. Gaston, A. Goldin)

With increased availability of "nude" (athymic) mice, a program has been initiated in an attempt to grow human tumors in these mice for chemotherapy and immunotherapy studies. In preliminary studies tumor material injected into the mice (obtained from the Clinical Center) included fresh ALL blood and bone marrow, ascites from an ovarian tumor patient, pleural fluid from a Burkitt's patient, adrenocortical carcinoma, and lymph node tissue from a breast tumor patient. Tissue cultured tumors included human lung and glioma. These tumors were administered by the subcutaneous and intraperitoneal routes and the mice are under observation for development of tumors. The mice have been observed from 2-7 months, but to date no progressive tumor growth has occurred. This program will be continued and studies will be undertaken to determine underlying factors that may influence the growth of allogeneic and xenogeneic tumors in the athymic mice.

F. Non-specific Immunostimulation: Comparison of BCG Preparation. (D. Houchens, E. Bonmassar, M. Gaston)

An assay to compare the effectiveness of various preparations of BCG was developed. The system involves prevention by immunostimulation of progressive growth of L1210 leukemia ($1x10^5$ cells) in BALB/c mice. The system was found to be reproducible, with the Tice and Connaught preparation being the most effective in this test, followed by Pasteur (fresh), Pasteur (freeze dried), Statens and Glaxo.

Further studies are in progress to determine the effects of BCG in a system where the animals are immunosuppressed before tumor transplantation.

G. Immunochemotherapy: Therapy Studies with a Murine Glioma Model.
(D. Houchens, L. Albright, J. Madigan, M. Gaston)

An intracerebral glioma tumor model was developed for immunotherapy and chemotherapy studies in conjunction with Drs. Albright and Madigan of NINDS and Dr. D. Young, Laboratory of Toxicology, NCI. This model employed the methylcholanthrene induced tumor GL-26 in C57Bl/6 mice. The subcutaneous tumors were grown in tissue culture in order to obtain large numbers of viable tumor cells for intracerebral challenge and for immunotherapy.

Tumor kinetics were determined _in vitro_ and _in vivo_ and dose-response survival experiments were conducted. Various combinations of pre-immunization, chemotherapy and immunotherapy were conducted. Tissue cultured cells were treated with mitomycin C, and _Vibrio cholera_ neuraminidase by the technique of Simmons to remove cell surface sialic acid and make the cells more immunogenic.

Preimmunization consisted of injection with these cells plus complete Freund's adjuvant. Chemotherapy was with one i.p. injection of CCNU (15 or 30 mg/kg) 3 days after intracerebral challenge. Immunotherapy consisted of neuraminidase treated cells plus viable BCG starting on day 3 after challenge and given every other day. Significant survival time increases were obtained with chemotherapy or preimmunization alone while immunotherapy alone caused a slight reduction of survival time compared to tumor controls, and may have actually caused enhancement of tumor growth.

III. Studies on Antigenic Changes of Tumor Cells Induced by Drug In Vivo
(E. Bonmassar, D. Houchens, M. Gaston)

A. Activity of DIC on Various Tumor Lines.

BALB/c and CDF_1 mice bearing LSTRA lymphoma were treated daily with DIC. Delayed tumor growth occurred in treated mice and tumor cells were collected and transplanted into syngeneic recipients. After 3-5 transplant generations the LSTRA/DIC subline became highly immunogenic in BALB/c mice and 10^6 cells were rejected by untreated control animals. Similar DIC-sublines derived from RBL-5 (MLV-induced lymphoma in mice of C57Bl/6 (_H-2_^{b}) strain) and K36 (spontaneous leukemia of mice of AKR (_H-2_^{k}) strain) and LPC-1 (plasma cell tumor of BALB/c (_H-2_^{d}) origin) are currently under study. These experiments are being conducted in order to determine whether the origin or the genetic make-up of the tumor could influence its susceptibility to DIC transformation.

B. Attempts to Utilize DIC-induced Antigenic Changes for Immunotherapy in the Primary Host.

CDF_1 or BALB/c mice preimmunized with viable cells of a DIC-transformed L1210 subline (L1210/DIC) were challenged with L1210 or LSTRA lymphomas and treated with DIC in order to induce antigenic changes in the host's tumor similar to those associated with L1210/DIC tumor. No protection was obtained in mice in which LSTRA lymphoma was transplanted, indicating that no therapeutically exploitable antigenic cross-reactivity occurred between the DIC-treated L1210 subline used for immunization and the transplanted LSTRA cells exposed to DIC treatment. On the contrary no leukemic deaths occurred in preimmunized CDF_1 mice transplanted with L1210 leukemia either untreated or treated with DIC. These studies provide evidence that lymphoma cell antigenically altered by DIC treatment and injected into mice compatible with the parental line, can protect the host against subsequent challenge with the same parent tumor cells not antigenically altered by DIC treatment.

Significance to Biomedical Research and the Program of the Institute:

The studies are part of a program to set up new strategies for the immunochemotherapy of malignant tumors. Attempts are being made to develop models suitable for studies of immunodepression induced by drug treatment and by tumor-associated factors in tumor-bearing hosts. Particularly a novel approach is being employed in an attempt to obtain new antigenic targets at the level of tumor cells through antigenic changes induced by drug treatment in animals transplanted with neoplastic cells.

Proposed Course of Project:

This section will be increasingly involved in searching for animal models for immunochemotherapy studies. In particular the new isotopic test will allow a better understanding and monitoring of antitumor-host reaction during treatment with antineoplastic agents alone or in combination with specific or non-specific immunotherapy. Intensive studies will also be conducted on drug-induced antigenic changes (DIAC) at the level of tumor cells, affecting classical histocompatibility antigens as well as antigenic properties under the control of Hh system. Efforts will be done to utilize DIAC for immunochemotherapy of mice carrying transplanted or spontaneous lymphomas. DIAC will also be tested in in vitro systems using animal or human tumor cells in order to obtain information on possible clinical applications. Combination of chemotherapeutic agents with specific or non-specific activators of the immune system will be studied.

A program will be continued to develop relevant models for measuring immunosuppressant action of antitumor agents and the relationship to chemotherapeutic response.

Athymic nude mice will be used to develop model systems to monitor chemotherapeutic and immunological responses to allogeneic or xenogeneic tumors either solid or of lymphoid origin with a view to potential clinical application.

Publications:

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3. Bonmassar, E., Menconi, E., Goldin, A., and Cudkowicz, G.: Escape of small numbers of allogeneic lymphoma cells from immune surveillance. J. Nat. Cancer Inst. In press.

4. Houchens, D. P., Goldberg, A. I., Gaston, M. R., Kende, M., and Goldin, A.: Studies of the effects of Bacillus Calmette-Guerin on Moloney sarcoma virus-induced tumors in normal and immunosuppressed mice. Cancer Res. 33: 685-690, 1973.

REPORT OF THE ASSOCIATE DIRECTOR
EXPERIMENTAL THERAPEUTICS

Experimental Therapeutics has major responsibility for an intramural research program and an extramural contract program concerned with the pharmacology, toxicology, and mode of action of cancer chemotherapeutic agents and other drugs of interest to the DCT. These studies cover a broad range of drug-host phenomena at the whole-body, cellular and molecular levels and are directed toward improving the chemical treatment of human malignancies, either by the use of single agents or in combined modalities. In addition, the program includes a major effort in molecular biology and biologic-biochemical markers. The former attempts to elucidate the physiological and cellular control mechanisms in normal and cancerous growth and to develop diagnostic markers and design of specific chemical inhibitors of the neoplastic transformation process. The latter program is aimed at quantitative measurements of residual tumor cell burden during and after therapy in an effort to improve therapeutic management of patients with cancer.

Toxicological studies

The Laboratory of Toxicology (LT) continues to have primary responsibility for providing the DCT program with animal pre-clinical toxicological information on antitumor drugs. This toxicological information is of value to the overall drug development program and also meets the FDA requirements for the submission of IND applications prior to clinical trial. In the past, this information was obtained by the laboratory's supervision of a number of contracts with various organizations. However, with program expansion and limited staff, the LT sought relief through the initiation of a Prime Contract which would share managerial responsibilities for the administration of protocol and special toxicological studies. Following an open competition, the Prime Contract was awarded to Battelle Memorial Institute during the past year.

Coupled with responsibility for the monitoring of protocol and special toxicological studies by contractors, various members of the laboratory are conducting basic research directed towards a fundamental understanding of the toxicity of anticancer agents. In an effort to elucidate pathologic effects of antineoplastic agents and in defining animal models of human diseases, the mechanism of L-asparaginase-induced hypocalcemia and adriamycin-induced cardiomyopathy continue to be studied in the rabbit. L-Asparaginase caused a specific lesion in the parathyroid glands of treated animals characterized by drug-endocrine cell interactions resembling the lethal selectivity of alloxan for pancreatic β cells in the induction of experimental diabetes. In addition to alteration of myocardial electrolytes and tissue fluids which precede the morphologic lesion in the hearts of adriamycin-treated rabbits, histopathologically demonstrable lesions were found in bones and kidneys. Whether such lesions precede congestive heart-failure in this anthracycline antibiotic-sensitive species remains to be determined.

In metabolic studies undertaken to elucidate the effects of microsomal and soluble enzymes of the liver on drug biotransformation and toxicity, significant differences were noted between the effects of adriamycin and daunomycin as well as between the nitrosoureas, BCNU, CCNU, and MeCCNU. Mixed function oxidase (MFO) activity was increased with daunomycin but decreased with adriamycin by about 25% in both cases for up to 2 1/2 weeks following a five-day course of treatment in mice. In patients with impaired hepatic function a higher degree of drug toxicity might occur than would be expected because of interference with adriamycin metabolism. The uniquely high degree of enhancement of MFO activity observed with MeCCNU however, might be associated with the lesser toxicity of this agent in large animals as compared to BCNU and CCNU because of increased metabolism.

Comparative biochemical toxicologic studies of L-asparaginase were continued during the past year. Such agents as 5-diazo-4-oxo-norvaline (DONV), 5-chloro-4-oxo-L-normaline (CONN) and 5-diazo-5-oxo-norleucine (DON) were found to have structural attributes of L-asparagine and of L-glutamine and the ability to inhibit L-asparagine synthetase. The most potent inhibitors of L-asparagine synthetase however, were two new agents, α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid and α-amino-3-chloro-4α-hydroxy-2-isoxazoline-5 acetic acid which might be useful for blocking the emergence of L-asparaginase resistance clinically.

Pharmacological studies

The elucidation of the qualitative and quantitative aspects of the physiological disposition and the mechanism of action of antitumor agents in man, laboratory animals and in vitro predictive systems continues to be the primary mission of the Laboratory of Chemical Pharmacology (LCP) intramural research and extramural contract programs. Particular attention is directed toward achieving a better understanding of the interrelationships of the pharmacokinetic behavior of antitumor agents between the biochemistry and cellular proliferative state of normal and cancerous tissue of the host. In addition, major emphasis is placed on the development of new methodologies where necessary to facilitate these studies. The information gained in these studies should improve our abilities to predict the therapeutic and toxic effects of drugs in man from their actions in animal models and provide the clinician with information for more effective use of these agents either through optimal scheduling or by modifications of chemical structure.

The folic acid antagonists, particularly methotrexate (MTX), have been the most intensively studied class of antitumor agents with regards to mechanism of action and disposition in the LCP during the last 15 years. The laboratory is recognized as a leading world authority in studies with these "model" agents. Although the antifolates, such as aminopterin and methotrexate, have been in widespread clinical use for almost a quarter of a century, such studies continue to provide us with valuable information on the general pharmacological principles of drug action and provide new clues for the more effective therapeutic use of these and other agents in human malignancies.

During the past year studies were undertaken to correlate plasma pharmacokinetics of methotrexate with the inhibition of DNA synthesis in critical normal target bone marrow and gastrointestinal epithelium as well as in tumor tissues of rodents as a means for allowing the design of optimal schedules. This could only be accomplished because the site and mechanism of action of MTX is sufficiently well understood so that the differences in the kinetics of recovering normal and tumor tissue can be measured and drug treatment schedules can be developed to take advantage of these differences. Interestingly, bone marrow of patients with ovarian cancer recovers from inhibition by MTX at plasma levels of 10^{-8}M or approximately the same threshold level as mouse bone marrow. This quantitative approach to determining the effects of drugs on normal and tumor tissue was also extended to 5-fluorouracil and was reported earlier for arabinosyl cytosine.

In collaborative clinical studies of the pharmacokinetics of MTX given intrathecally for patient therapy, comparison of cerebrospinal fluid antifolate levels with body surface area, age and disease state provided information for adjusting dosage levels to lower the incidence of neurotoxicity.

Lipid soluble dialkyl esters of MTX continue to be examined for their antitumor activity, effects on dihydrofolate reductase, and pharmacological disposition. Such agents are of potential usefulness for the treatment of meningeal leukemia when the drugs are given systemically.

In vitro studies of the cellular transport kinetics of MTX in animal tumor systems were extended to human AML and ALL cells and normal marrow during the past year. While no apparent differences were observed between ALL cells and AML cells with respect to MTX transport kinetics, MTX levels within these cells were enhanced by concomitant use of readily achievable plasma levels of vincristine and reduced by both hydrocortisone and cephalothin. These studies are directed toward elucidating alterations in cellular transport and ultimately antitumor activity of MTX in various human malignancies when this drug is used concurrently with other cancer chemotherapeutic agents and antibiotics.

The mechanism of selectivity of action of 5-methyl-d,l-tetrahydrohomofolate against antifolate-resistant variants of the murine L1210 leukemia was studied during the past year and results indicate that this agent exerts its antitumor effect by mechanism(s) not shared by other antifols such as MTX.

Considerable effort was placed in pharmacological studies of agents which have either not yet reached clinical trial or are in early evaluation in patients with cancer. The absorption, distribution, mechanism and excretion of the following agents were undertaken or continued during the past year: 2,6-dimethyl-4-benzyl-4-demethylrifamycin, the acridine derivative NSC-141,549, inosine dialdehyde NSC-118,994,DL-alanosine, quinaldine, and cyclocytidine.

The lymphatic absorption and distribution of various model antitumor drugs such as guanazole, dibromomannitol, diethylstilbesterol, estradiol and testosterone were studied in the rat. It is expected that when these studies are complete, clues will be provided for enhancing the absorption and distribution of antitumor agents by lymphatic channels, an important route in addition to blood for the spread of tumor metastases.

One of the most significant developments during the past year in the area of biochemical pharmacological studies was the development of a new highly sensitive and specific enzymatic assay for 5-FdUMP. The assay now opens the way for comparing the pharmacokinetic behavior of 5-FU with the active 5-FdUMP metabolite in animal systems and in patients with cancer. Such studies should provide additional biochemical and pharmacological rationale for optimizing the use of 5-FU clinically.

In addition to thymidylate synthetase used in the FdUMP assay, several enzymes involved in the metabolic activation or catabolism of antitumor agents were studied this past year. This includes aldehyde oxidase, xanthine oxidase, cytidine deaminase and cytidine kinase. Following extensive purification, the latter two enzymes are being studied to define their biochemical role in the responsiveness of sensitive and resistant human leukemic cells (AML) to arabinosyl cytosine. The mechanism of the inhibition by a series of acridine derivatives and other N-heterocyclic antitumor agents of the oxidase enzymes is also being investigated.

The Membrane Transport Section continues to take a leading role in studies of the transport of various physiological and pharmacological substances between blood, brain, and cerebrospinal fluid (CSF) in sub-human primates, and lower vertebrate species. This work is expected to form the basis for developing the principles which are involved in the exchange of materials between blood, brain, and CSF and the guidelines which should be used in planning optimal route and dosage schedules for the uptake and distribution of drugs to normal and neoplastic CNS tissue.

Molecular biology and pharmacology studies

The major thrust of the research program of the Laboratory of Tumor Cell Biology (LTCB) is directed towards gaining a better understanding of the processes involved in the molecular control of cellular growth and differentiation, neoplastic transformation, and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for therapy of human malignancies. Intimately related to this program is the search for and development of markers of neoplastic growth which give a quantitative assessment of residual tumor cells after therapy.

The above objectives have been pursued primarily in studies of a) the purification and properties of enzymes of nucleic acid biosynthesis, particularly the DNA polymerases (DNA-dependent and RNA-dependent) of human normal and leukemic cells and of RNA oncogenic viruses; b) the biochemical properties of RNA and type-C viruses with special emphasis on determining the mechanism of transcription of RNA to DNA by reverse transcriptase; c) the origin and oncogenic potential of tumor viruses; d) leukocyte differentiation *in vitro* using the soft agar technique of Paran and Sachs; e) mechanism(s) of the initiation of DNA synthesis in normal and tumor cells with the development of selective DNA polymerase inhibitors to be evaluated in human normal and leukemic cells; and f) immunochemical techniques for detecting reverse transcriptase in intact cells and other techniques for detection of viral specific nucleic acids in intact cells.

A number of major findings were reported by the Laboratory during the past year, but only a few are highlighted. A DNA polymerase (reverse transcriptase) was found in human leukemic lymphocytes which can catalyze DNA synthesis from an RNA template and which does not have the properties of DNA polymerase from normal human lymphocytes. In a collaborative effort with the Viral Leukemia Lymphoma Branch, NCI, it was demonstrated that this reverse transcriptase found in many cases of human myelogenous leukemia is immunologically closely related to reverse transcriptase from type-C viruses isolated from tumors of sub-human primates. Evidence was also presented which showed that the major DNA polymerase of normal human lymphocytes is not immunologically related to reverse transcriptase of known viruses or reverse transcriptase from human leukemic cells. It was further demonstrated that chronic lymphocytic leukemia cells contain a reverse transcriptase-like enzyme which is not related to reverse transcriptase of known RNA tumor viruses. The development of new and improved techniques for the rapid isolation, purification, and characterization of all known DNA polymerases from normal, type-C virus infected and leukemic human leukocytes from relatively small quantities of cells were highly instrumental in achieving these and other findings.

In the Laboratory of Molecular Pharmacology a new technique, alkaline elution analysis, was further studied to detect the effects of X-ray, ultraviolet light, and certain antitumor drugs on DNA strand growth and joining during recovery from replication block. These studies, which were carried out *in vitro* primarily in L1210 leukemia cells and in HeLa cells and normal human fibroblasts, are aimed at understanding the mechanism of cell killing by inhibitors of DNA synthesis as well as to determine possible differences between normal and neoplastic cells in their responses and ability to recover from drug or radiation treatment. Of twenty chemical agents studied, fourteen produced measurable strand breakage but only BCNU, CCNU and their isocyanate degradation products (presumably formed *in vivo*) were capable of inhibiting the rejoining of X-ray-induced DNA single-strand breaks in L1210 cells. Contrary to earlier reports from elsewhere, bleomycin does not inhibit repair of DNA breaks after x-irradiation.

Biologic markers program

Initial efforts focused on the development of specific and sensitive methods for determining potential biologic markers and in evaluating these materials utilizing body fluid specimens from patients with advanced malignant disease. Preliminary emphasis has centered on compounds present in urine and or blood including tRNA minor base degradation products (methylated nucleosides:N^2,N^2-dimethylguanosine and 1-methylinosine, and pseudouridine) and polyamines (putrescine, cadaverine, spermidine and spermine). These compounds have been studied using such methods as gas-liquid chromatography, high-pressure liquid chromatography, and the amino-acid analyzer. Elevations (above two standard deviations) compared to normal control urines have been found for patients with solid tumors as well as for patients with specific hematologic malignancies.

It is a basic premise in the program that multiple markers should be evaluated for each patient admitted to the studies. By these means it is anticipated that 1) a greater number of patients with a specific malignancy will be identified with a marker, 2) comparisons between markers and their utility can be made, 3) patterns of markers will be found for various tumor types and 4) a possible better understanding of laboratory manifestations and abnormal processes of these diseases will be gained. Consequently, several potential markers are undergoing studies in the same patients either routinely or for specific purposes. In addition to those mentioned, other markers include CEA, HCG, αFP, Regan's isoenzyme, serum fucose/protein ratio, specific β-aminoacids (β-aminoisobutyric acid, β-alanine, etc.), urinary hydroxyproline/creatine ratio and various trace metals. A urine-serum bank has been established so that future markers can also be determined and comparisons made with previous analyses. Clinical studies include patients in the various stages of breast cancer, Phase II chemotherapy studies in patients with solid tumors, and the monitoring of selected patients during therapeutic trials. Diseases included in these studies are lung, breast, ovarian, colon and other GI tumors, melanoma and Burkitt's lymphoma.

Animal studies are in process to develop and study specific animal systems as models for human malignancy. Considerable effort and progress has been made in defining α-FP as a marker in subhuman primates with chemical-induced hepatomas. Studies in the development of other types of tumors (e.g. lung, GI malignancies in similar subhuman primates) with available markers are in process. The use of these monkey systems, and other animal models are being investigated as a means for establishing methods for determining total tumor mass.

Serial No. NCI-3500
1. Office of the Associate Director for Experimental Therapeutics
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Distribution of Drugs Between Blood, Brain, and Cerebrospinal Fluid

Previous Serial Number: Same

Principal Investigator: Joseph D. Fenstermacher

Other Investigators: Ronald Blasberg, Walter Eckman, Warren Lux, John Kessler, Alan Cowles, Clifford Patlak, Anthony Guarino, David Prieur, Leon Sokoloff, Ruth Geran, Arnost Kleinzeller (non-NIH), Andres Roomet (non-NIH), and James Ausman (non-NIH)

Cooperating Units: Biometrics Branch, NIMH; Laboratory of Cerebral Metabolism, NIMH; Laboratory of Toxicology, DCT/NCI; Drug Evaluation Branch, NCI; Department of Physiology, University of Pennsylvania; Department of Neurology, University of Vermont; Department of Neurosurgery, University of Minnesota

Man Years

Total:	6.5
Professional:	4.0
Other:	2.5

Project Description

Objectives:

The objectives of this project are to quantify the transport of various materials of physiological and pharmacological importance between blood, brain, and cerebrospinal fluid (CSF) and to study the effects of various pharmacological agents and pathological conditions upon the transport and metabolism of essential materials, e.g. amino acids, within the blood-brain-CSF system. It is the purpose of such studies to characterize the transport properties of the blood-brain-CSF system and to develop methods for obtaining more effective central nervous system (CNS) concentrations of antitumor drugs.

Methods Employed:

The method of ventriculocisternal perfusion of various radioactive materials and subsequent brain tissue sampling has been utilized in monkeys, dogs, and dogfish sharks for measurements of brain capillary and cellular permeability and CSF production and reabsorption. A constant pressure flow system has been employed for studies of the CSF absorption capacity in elasmobranchs. An experimental system employing simultaneous intracarotid and intravenous infusions has been devised and used for assessing the effectiveness of these routes of drug administration. Studies of brain and CSF uptake and distribution of intravenously administered, radioactively labeled physiological and pharmacological materials have been undertaken in dogs, monkeys, and elasmobranchs. Finally, a technique for studying the growth of regenerating optic nerves and brain (optic tectum) in goldfish is being developed.

Major Findings:

An examination of the exchange of material between the CSF and the brain and spinal cord of monkeys was made using our technique of ventriculo-cisterno-lumbar perfusion. From this work we determined that the permeability of the spinal cord's pia-glial covering to sucrose and EDTA-Ca was similar to that of the brain's ependymal and pia-glial membranes, and that these molecules exchange readily between CSF and spinal cord. Using the equilibrium sucrose and EDTA-Ca spinal cord to CSF ratios (they were virtually identical) an extracellular space (ECS) of 12% was measured for the monkey spinal cord. The rates of tracer compound equilibration within the spinal cord's ECS were comparable with the diffusional exchanges previously reported from this laboratory for the caudate nucleus and cerebral cortex. Using this same technique, CSF production by periventricular brain tissue and spinal cord were measured by blue dextran dilution. The results indicate that the rate of CSF formation in the monkey is about 30 μl/min and that little or no CSF is produced by the spinal cord.

Our theoretical, computer-modelling, and experimental studies of the CNS tissue distribution of drugs following either intravenous or intraarterial administration indicate that the latter route of administration will only be advantageous for very specific circumstances. Antineoplastic agents should be infused by the arterial route, usually the carotid artery, when it is important to achieve a high initial or peak concentration in the brain, when the drug has a high affinity for the target organ or for all tissues, or when the drug is rapidly metabolized. These drugs should be given intravenously when the compound has a low affinity for the target organ, is metabolized slowly, or equilibrates slowly between capillary blood and the cells of the target organ; in addition this route should be used when local tissue toxicity precludes arterial infusion. From this work we have shown that the considerable advantage of increased drug delivery to the CNS obtained during the initial or first passage of arterially injected drug is rapidly dissipated if the material continues to recirculate in significant amounts, even for relatively short periods of time.

Using a double-indicator (tri-iodo-fluoro-methane and antipyrine) uptake technique, regional differences in blood flow and capillary permeability have been found within the CNS of the cat. This work shows that significantly higher (3-5 times) rates of blood flow occur in areas such as the cerebral and cerebellar cortex and caudate nucleus than in other regions such as the pons, periventricular white matter, and spinal cord. For highly permeable compounds sizeable regional differences in the kinetics of molecular exchange between blood and tissue exist because of these differences in the rates of blood flow. From these results plus some of our ventriculocisternal perfusion studies, we are also finding some differences in capillary permeability (the blood-brain barrier) between various areas of the CNS. In addition, attempts to experimentally increase the permeability of the blood-brain barrier by various membrane active agents and hyperthermia were made. The agents used were vitamin A and amphotericin-B; the temperatures ranged from 41-45°C. No detectable changes in the permeability of the blood-brain barrier to mannitol and urea were found when sublethal concentrations or temperatures were used.

A quantitative study of the uptake of an extracellular marker, inulin, and a vascular marker, albumin was made in brain tumor, brain adjacent to tumor, and distant brain. The choriocarcinoma, intracerebrally transplanted and growing in rhesus monkeys, was used as the brain tumor model. Significantly larger vascular spaces were found in the tumor than in the other two sampling sites. The extravascular inulin space in the tumor was 24%, whereas the normal brain's extravascular inulin space was 0.6%. Kinetic analysis indicated that inulin entry into the tumor was unrestricted as in muscle and that the ECS of the tumor was considerably larger than the ECS of normal CNS tissue. The distribution of inulin away from the tumor site was found to be more compatible with diffusion from the tumor than with increased blood-brain barrier permeability of the tissue surrounding the tumor. A preliminary study of the possible correlation between antitumor activity (using the mouse ependymoblastoma model) of 15 chemotherapeutic agents and their ability to be taken up by the brain and CSF from the blood was made. The brain and CSF exchange data were obtained in dogfish sharks at two time intervals, 4 and 24 hours, after intravenous injection. The correlation between brain and CSF drug concentration and effectiveness against the model tumor system showed a good correlation for the effective drugs; however a significant number of the ineffective drugs also achieved relatively high tissue and fluid levels. Although adequate delivery of the drug to the CNS and CSF is necessary for the effective treatment of brain tumors and must be considered when attempting to choose drugs for chemotherapeutic trials, pharmacological and biochemical factors must also be taken into account.

From our experimental and theoretical studies, we are formulating the principles which are involved in the exchange of materials between blood, brain, and CSF and the guidelines which should be used in planning optimal route and dose schedules for the uptake and distribution of drugs to normal and neoplastic CNS tissue. Although the capillaries within the core of the tumor seem to be highly permeable to drugs, the growing edge apparently possesses a relatively normal blood-brain barrier. It is the latter portion of the "tumor" to which the anti-tumor agents must be delivered, and thus, the cancer chemotherapist must select compounds which cross the blood-brain barrier. To obtain the most uniform and widespread distribution of drug to the

CNS as well as the CSF and meninges, the material should be introduced into blood stream; yet for this route of delivery to provide adequate tissue uptake, the drug must be either lipid soluble enough to passively diffuse across the blood-brain barrier or structurally resemble one of the types of molecules which are transported across the blood-brain barrier by special (carrier) systems. On the other hand, compounds which do not readily cross the blood-brain barrier may be delivered to the meninges and surface portions of the brain and spinal cord by way of the CSF. There are, however, significant problems in drug delivery such as adequate mixing throughout the CSF compartment and depth of tissue penetration when this route of drug administration is used. Before infusing the drug into the CSF, consideration must be given to the site and manner of administration, the location of the tissue target site, and the transport properties of the compound.

From our comparative studies, a wide range of observations have been made. Neoplasms of elasmobranchs are quite rare and no tumors of the CNS have been previously reported in this class of animals. During the course of our work at the Mount Desert Island Biological Laboratory, a peculiar growth was found in the fourth ventricle choroid plexus of a dogfish shark. After suitable preparation for light and electron microscopy and analysis, we concluded that this is a benign neoplasm arising from the choroid plexus which can be best described by the general term ependymoma. Ventricular fluid (CSF) production rates were measured by ventricular perfusion on the spiny dogfish and found to be about .002 ml/min. In this species the resistance to bulk absorption of CSF was determined to be similar to that in mammalian species such as the rabbit and monkey; the cranial nerves and spinal canal seem to be the major pathways of CSF absorption. Using the in vitro choroid plexus preparation of Rall and Sheldon, the kinetics of glucose transport of this tissue were studied. Evidence of transport saturation, stereospecificity, and competition were found. The movements of a series of polar compounds from blood to CSF and several general regions of the CNS were examined in the dogfish shark. From the water exchange data rates of blood flow were found ranging from 2.2 ml/min-gm tissue for the choroid plexus to 0.04 ml/min-gm tissue for the spinal cord. The movement of urea from blood to CSF was surprisingly rapid and suggested that the flow of this compound between these two fluids is mediated by a special transport mechanism located at the choroid plexuses.

Significance to Bio-medical Research and the Program of the Institute:

Tumors, both primary and metastatic, frequently develop in the brain, spinal cord, and meninges. Anti-tumor agents can be delivered to these tissues by the vascular and/or the cerebrospinal fluid systems; however, each of these two systems appears to have certain limitations as a means of effective drug delivery. Basic information concerning the transport physiology and movement of material into normal and neoplastic CNS and meningeal tissue from the blood and from the CSF is needed for the design and development of effective brain tumor chemotherapy protocols. Such information about blood-brain-CSF transport coupled with a knowledge of the physiochemical and pharmacological properties of specific antitumor drugs will enable the chemotherapist to choose the best route, manner, and timing for effective drug delivery to CNS and meningeal lesions. Basic knowledge of the transport of ions, metabolites, and other materials between blood, brain and CSF is essential to the understanding of the activities and functions of the central nervous system. Since our work is fundamental in nature, it is relevant to work being carried out by many other groups of bio-medical researchers, and important for an understanding of both normal and pathological brain and spinal cord processes. The type of work performed in our laboratory on this subject is, thus, meaningful and significant to the neurologist, the neuroncologist, the neurosurgeon, and the neuropathologist.

Proposed Course:

1. Continue our studies of the transport of various physiological and pharmacological materials between blood, brain, and CSF and more precisely quantitate the permeability of brain capillary and cellular membranes.

2. Investigate further the formation, flow, and absorption of cerebrospinal fluid by and through CNS tissue.

3. Examine the effects of high and low dietary levels of vitamin A on CSF production, absorption, and pressure and on brain cell and capillary permeability of the rhesus monkey.

4. Determine the effects of several steroids on blood, brain, and CSF electrolytes, the blood to CSF electrical potential, and blood-CSF-brain exchange of water and ions.

5. Study regeneration, control systems, and growth factor(s) in the goldfish central nervous system.

6. Continue to examine the intra-axonal flow of materials in goldfish cranial nerves and lobster spinal cord.

7. Physiologically and histologically analyze the phylogeny of the blood-brain barrier in lower vertebrates.

8. Further examine the peculiar blood to CSF movement of urea and other amides in elasmobranchs.

9. Continue our study of the transport of organic compounds by the dogfish choroid plexus *in vitro*.

10. Investigate the effects of various transport inhibitors such as amiloride and phloretin on the blood-brain-CSF exchange of ions and amides in elasmobranchs.

Publications:

1. Cowles, A. L., and Fenstermacher, J. D.: Theoretical considerations

in the chemotherapy of brain tumors. In Sartorelli, A. G. and Johns D. G. (eds.): Handbuch der Experimentellen Pharmakologie (Antineoplastic and Immunosuppressive Agents). Berlin, Springer-Verlag (in press).

2. Fenstermacher, J. D., and Blasberg, R. G.: Pharmacological considerations in the chemotherapy of central nervous system tumors. Biochim. Pharmacol. (in press).

3. Fenstermacher, J. D., Patlak, C. S., and Blasberg, R. G. The transport of material between brain extracellular fluid, cells, and blood. Fed. Proc. (in press).

Serial No. NCI-3516
1. Office of Assoc. Dir. for Exp. Ther.
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemical, Pharmacologic, Toxicologic and Chemotherapeutic Aspects of Selected Antitumor Agents

Previous Serial Number: Same

Principal Investigator: J.A.R. Mead

Other Investigators: V. T. Oliverio and M. C. Castle, NCI; A. E. Auletta, R. Tomchick and L. C. Mishra, Microbiological Associates, Inc.;J. P. Kutney, University of British Columbia, and W. A. Bleyer

Cooperating Units: None

Man Years

Total:	4
Professional:	2
Other:	2

Project Description:

Objectives:

To investigate the biochemical pharmacology and pharmacodynamics of new or established antitumor agents to better define the important parameters related to their chemotherapeutic activity.

Methods Employed:

Standard laboratory procedures were employed.

Major Findings:

The studies in this project can be categorized under the following headings:

A. Homofolate Derivatives
B. Quinaldine
C. Cyclocytidine
D. Vincristine

A. Homofolate Derivatives

Studies with 5-methyl-d,l-tetrahydrohomofolate. The selectivity of action of this agent against antifolate-resistant variants of L1210 has been investigated using radioactive precursors of DNA synthesis such as thymidine, deoxyuridine and formate. The effect of the drug at therapeutically active dose-levels on the incorporation of these precursors into DNA has been studied in bone marrow, spleen, gut lining and tumor from mice bearing L1210 and the antifolate-resistant variant L1210/FR-8. The incorporation of all three precursors was only marginally affected by the drug in all the systems examined. This lack of biochemical effect in systems known to be sensitive to other antifolates such as methotrexate indicate that this agent exerts its antitumor effect via some mechanism, as yet undefined, which sets it clearly apart from the usual antifols.

In another study, three attempts have been made to develop a tumor resistant to 5-methyl-tetrahydrohomofolate by treating leukemia L1210 with daily doses of 400 mg/kg of the drug. The purpose of the study was to see if resistance to methotrexate developed concomitantly or whether the response to the two drugs was unrelated. In each of the attempts the tumor retained its original level of sensitivity to the drug and even after 12 treated transplant generations there was no loss of sensitivity to methotrexate. No change in dihydrofolate reductase levels were observed during this treatment period. Attempts will now be made to see whether the more sensitive L1210/FR-8 tumor can be made resistant to 5-methyltetrahydrohomofolate and if so whether there is any change in responsiveness to methotrexate.

In another study the conversion of dihydrohomofolate to tetrahydrohomofolate was established in vivo both in leukemia L1210 and the antifolate resistant L1210/FR-8 characterized by high levels of dihydrofolate reductase. These findings were in contrast to those of others but was clearly established and showed also that greater amounts of tetrahydrohomofolate were formed in the enzyme-enriched tumor and could thus account for the greater antitumor effect observed against this tumor.

B. Quinaldine

This drug which is quite active against leukemia L1210 is presently under development in the program. There are some problems with its formulation and it is administered to mice in suspension. A spectrophotometric assay procedure was developed and a physiological distribution study was performed in mice. Little drug was excreted in either urine or feces. The drug was found in most tissues, particularly the spleen, but was only very slowly cleared from the animal. In fact as long as 90 days after administration appreciable amounts of unchanged drug were still present in the animals. The total recovery of drug was poor after i.p. administration suggesting that some metabolism had occurred which was not detected using the spectrophotometric method. Since the drug is quite active given as a single dose and since its presence in vivo is very persistent, the duration of its antileukemic potency was determined by giving the drug at its maximum tolerated dose at varying times before inoculation of leukemia L1210. The antileukemic effect dropped to zero after about 24 hrs although large amounts of drug were still evident. It appears that bioavailability is a serious problem which will have to be solved before clinical trial is possible.

Very recently a water-soluble acetate derivative of this agent has been prepared. We have examined the distribution and excretion of this derivative and have found that it behaves very similarly to the drug when given in suspension. We would predict therefore that this formulation will have similar antitumor effects to the parent drug when tested in vivo.

C. Cyclocytidine

In the period before his untimely death, Mr. R. Tomchick was able to establish quite clearly that although there was an appreciable accumulation of radioactivity in the salivary glands of mice after administration of the labeled drug, it was not due to ara-C or its nucleotides but rather to unchanged cyclocytidine. Since parotid pain is a commonly observed clinical effect of the drug, it suggests quite strongly that it possesses pharmacological properties which appear to be unrelated to its conversion to cytosine arabinoside and its subsequent phosphorylation. The salivary glands, it must be remembered, exhibit a remarkable response to isoproterenol and since a postural hypotension has also been observed clinically after its administration of cyclocytidine an autonomic effect might be suspected.

D. Vincristine

In a collaborative study with Dr. James Kutney of the University of British Columbia we have mounted a study of the metabolic disposition of vincristine using specifically labeled vincristine of greater than 95% radiopurity. Excretion studies in the bile feces and urine of rats given the labeled drug indicated a half-life of approximately 48 hrs. The major route of excretion appears to be the bile and this will be used as a source for isolation and characterization of metabolites. Preliminary evidence suggests that more than two-thirds of the label in bile is in the form of metabolites. At least four discrete components can be recognized with the help of high pressure liquid chromatography. This technique will be used to refine the separation and isolation of these metabolites in sufficient quantity to permit identification.

Uptake, metabolism and binding of tritium-labeled vincristine sulfate were studied in L1210, P388 and P388/VCR murine leukemia cells in vitro. Negligible metabolism of the drug by these cells was suggested by thin-layer chromatography experiments. Accumulation of total and of bound vincristine occurred most rapidly in P388 cells, the subline most sensitive among those studied to the cytotoxic effects of vincristine. The results implicate the presence of an active transport mechanism for translocation of the drug into the cells, and suggest that drug resistance in these cells is due at least in part to impaired accumulation and binding of vincristine within the cell.

Proposed Course of Project

The project will continue to follow a course aimed towards the acquisition of relevant pharmacological, toxicological and biochemical data for input into the Linear Array for drug development. Concurrently, phenomena of a basic nature will be investigated inasmuch as they are related to the physiological effects, in animals, of experimental antitumor agents.

Honors and Awards: None

Publications:

Mishra, L.C., and Mead, J.A.R.: Physiologic disposition of 1-acetyl-2-picolinoylhydrazine in rats bearing Walker carcinosarcoma 256. Cancer Res. 33: 2393-2397, 1973.

Mishra, L.C., Parmar, A.S., and Mead, J.A.R.: Metabolism of tetrahydrohomofolate (NSC-89473) in mouse. Cancer Res. (in press).

Mishra, L.C., Parmar, A.S., and Mead, J.A.R.: Regeneration of tetrahydrohomofolate in cells: Possible basis for antitumor activity of homofolates. Biochem. Pharmacol. (in press).

Auletta, A.E., Gery, A.M., Parmar, A., Davis, J., Mishra, L.C., and Mead, J.A.R.: The effect of folate and folate analogs upon biochemical events in kidneys of normal mice. Life Sciences (in press).

Auletta, A.E., Gery, A.M., and Mead, J.A.R.: Influence of anti-leukemic (L1210) treatment schedule on disposition of (-)-emetine hydrochloride (NSC-33669) in normal and leukemic mice. Cancer Res. (in press).

Mead, J.A.R.: Biochemical pharmacology and drug design; in pharmacological basis of cancer chemotherapy. Proceedings of the 27th Annual Symposium on Fundamental Cancer Research, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas (in press).

SUMMARY REPORT

LABORATORY OF TOXICOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1973 to June 30, 1974

I. Introduction

Since its inception five years ago, the Laboratory of Toxicology has retained the responsibility for supervision of large animal toxicologic studies of antineoplastic agents prior to their introduction into man. This important role has actually been carried out in the past by a rather diffuse contract mechanism, with the Laboratory of Toxicology retaining responsibility to direct these studies, summarize the information, and aid in the submission of an INDA to the Food and Drug Administration before a Phase I Clinical Trial is initiated. In February of 1974, the Division awarded a prime contract, wherein the responsibility for these studies will be shared by the Prime Contractor (Battelle Memorial Institute) and this Laboratory. It is the expectation that this Prime Contract will make available more time for the inhouse staff to more properly pursue some important aspects of research into toxicological mechanisms of action caused by antineoplastic agents. Hence, for the next fiscal year the Laboratory is committed to two major goals: 1) to continue the improvement of the inhouse staff efforts in the area of basic research; and 2) to cooperate with the Prime Contractor in getting this effort off to a good start. It is felt that with the current limitations of staff, it would be unrealistic to project any additional goals. On the other hand, if staff conditions are improved it is the strong desire of the Laboratory to define and support research in the area of delineating mechanisms of toxicity of anticancer agents with the hope of proposing remedies for these toxic actions.

As was mentioned in last year's annual report, a new level of excellence in basic research was sought on the part of the senior staff of the Laboratory of Toxicology. These efforts have only been partially successful, primarily due to the loss of about 20% of our positions over the past year. Attempts to sustain research efforts in the face of such personnel losses have been accomplished by some investigators only by heroic personal effort. While these efforts are appreciated, it is unfair and unhealthy to expect this to continue for the next fiscal year. We have attempted to help this situation in every way possible. For example, redistribution of the contract responsibilities has allowed for more research time for most individuals. For some individuals, the efforts involved over the past year in the solicitation and award of the prime contract has seriously cut into their research time. On the other hand, because of delays in awarding the prime contract some individuals were able to continue their research unincumbered for several months by contract responsibilities. Finally, it must be pointed out that the Prime Contract will take a different kind of effort because of the "incentive" features of it. In other words, because this contractor will be receiving a portion of his fee from this mechanism, extensive documentation is required to justify the award or denial of this fee. It has been very difficult to structure the roles of the members of the Laboratory in this award-fee-situation. It has been recommended that there should not be overlapping roles between the persons requesting the work and the individuals recommending the award of funds as judged by performance of this same work. Thus three categories of personnel are required for proper operation of this prime contract: 1) compound officers, 2) contract monitors, and 3) evaluators. Hence, it should be obvious that with four to five senior staff slots, the requirements that the three roles not be overlapping is going to be difficult, if not impossible, to meet.

We continue to insist, as we have in the last annual report, that the supervision of contractors should be done by members of the scientific staff who have considerable first hand knowledge about the research problems involved with the use of our often uniquely toxic antineoplastic agents. If the internal staff does not maintain this kind of knowledge, then some important observations by contractors can not be properly assessed by individuals lacking an adequately "prepared mind". For example, two groups in the Laboratory felt that there were serious gaps in two areas involved with Adriamycin toxicity. Hence, while some contract effort is being expended in defining the cardiac toxicity of this agent, the possible involvement of other organs which may have primary or secondary etiologic contributions, has been investigated. Thus alterations have been demonstrated by the inhouse staff implicating kidney, bone, intestine, and liver as possibly contributory to the cardiotoxicity of Adriamycin. The degree of emphasis on basic research prowess represents a turn up a new road as far as the Laboratory is concerned and during this second year of such a policy it would seem that definite progress has been made. Evidence for this will be seen in the individual reports following, where it will be particularly noted that a number of projects have been brought to logical conclusions and submitted for publication.

II. Internal Research Programs

The independent projects of the senior staff members are primarily directed toward attempting to understand the actions of antineoplastic agents, especially those actions which manifest themselves as some form of toxicity. It is only through an understanding of such actions that we will be able to advise clinicians intelligently as to what toxicity to expect, and when toxicity does occur, what steps may be taken to reverse this.

A. Biochemistry Unit

The Biochemistry Unit is engaged in several projects related to the properties of oncolytic enzymes and involving the mechanisms of toxicity of selected nitrosoureas. Comparative biochemical, pharmacologic and toxicologic studies have been conducted with 5-diazo-4-oxo-L-norvaline (DONV), 5-chloro-4-oxo-L-norvaline (CONV) and 6-diazo-5-oxo-L-norleucine (DON). Structurally these drugs have attributes of L-asparagine and of L-glutamine. All inhibit L-asparagine synthetase significantly. However only DON and CONV inhibit other amido transferases. In comparative studies in mice, it was found that CONV was the most toxic while DONV was the least toxic. In studies with isolated cells of Leukemia 5178Y, all three agents were found to compete both with L-asparagine and L-glutamine for transport into the cells, but DONV competed most effectively with L-glutamine and CONV most effectively with L-asparagine. It was concluded that DON is the most "L-glutamine-like" agent of the trio while DONV is the most "L-asparagine-like". After years of searching for potent inhibitors of L-asparagine synthetase, two were found this year; these are NSC-163,501 (5-isoxazoleacetic acid, α-amino-3-chloro-4,5-dihydro-) and NSC-176,324 (2-isoxazoline-5-acetic acid, α-amino-3-chloro-4α-hydroxy. These compounds were screened as part of the Biochemistry Unit's program to find inhibitors that would block the emergence of L-asparaginase resistance by inhibition of L-asparagine synthetase. Another group of analogues, ethacrynic acid and related compounds, was tested for similar inhibition. These were found to block the utilization of ammonia by L-asparagine synthetase to a much greater degree than the utilization of L-glutamine. A third class of agents examined as inhibitors of L-asparagine synthetase was mucobromic and mucochloric acids. These acids also produced highly selective inhibition of the utilization of ammonia by the enzyme. The Biochemistry Unit has continued its efforts to improve the analytical methodology required in its research programs. For example, because of the long hours required for the analysis of L-asparagine synthetase, revised methods for accomplishing the assays have been developed. Also, this Unit has developed an analytical method for separating ATP from pyrophosphate on the basis of differential solubility in 80% ethanol. This methodology will have application in studying the enzymatic mechanisms involved in L-asparagine synthetase actions. In a third area of activity, studies of oncolytic enzymes have been continued. Because of the long experience of this Unit with L-asparaginase, they have recently begun to study L-phenylalanine-ammonia lyase (PAL). These studies are barely underway since the first batches of the enzyme were not regarded to be of sufficient purity for extensive studies. In a fourth research area, the Biochemistry Unit has been investigating the methylation of purine residues by Streptozotocin. Recent availability of [^{14}C]-Streptozotocin allowed these investigations and it was found that 7-methyl-guanine was detectable in organs of Streptozotocin treated mice. This methylated purine also was found in rat nesidioblastomas which had developed as a consequence of the co-carcinogenic action of nicotinamide and Streptozotocin. Since nicotinamide reverses the diabetic effects of Streptozotocin, it is also of interest to investigate whether this vitamin would diminish alkylation of DNA. If this were true, then such studies would elucidate the biochemical mechanism of toxicity.

B. Comparative Pathology Unit

One of the more important achievements of the Comparative Pathology Unit has been in the area of providing methodology of tabulating and eventually computerizing quantitative relationships of toxicity of antineoplastic agents in dogs and monkeys, and further, to correlate such findings with the toxicity studies done at the Clinical Center. Personnel in this Unit are expected to continue to work closely with the Prime Contractor in order to provide a mechanism by which a clinician could ask whether a given side effect has occurred in humans or in any of the large animal species studied to date. The primary research efforts of this group have been in the area of elucidating pathologic effects of antineoplastic agents and in defining animal models of human diseases. The studies of the mechanism of L-asparaginase-induced hypocalcemia in rabbits have been extended and concluded during the past fiscal year. The major finding of this study was that L-asparaginase caused a rather specific lesion in the parathyroid glands of animals treated with this oncolytic enzyme. Further studies ruled out uremia and contaminating endotoxin as possible etiologic factors in the development of the L-asparaginase-induced hypocalcemia and tetany. This model of drug induced hypoparathyroidism was characterized by drug-endocrine cell interactions somewhat akin to the lethal selectivity of alloxan for pancreatic β cells in the production of experimental diabetes. In other studies of the effects of Adriamycin in causing cardiomyopathy in rabbits, it was found that conventional animals were carrying too large a burden of pathogenic microorganisms to allow the conduct of such studies. Therefore, the present efforts employ the use of specific pathogen free (SPF) rabbits. It was found that intravenous administration of Adriamycin to SPF rabbits at a dose schedule of 7.7 mg/M^2 three times a week resulted in a 100% incidence of cardiotoxicity. These studies revealed that rabbits, receiving total doses of 254 - 416 mg/M^2, had evidence of cardiomyopathy indicating that the rabbit is more sensitive than man since the latter is reported to tolerate 500 mg/M^2. By the measurement of mineral levels in the myocardium, it was found that there were significant increases in calcium and sodium concentrations in the heart muscle compared with control animals. By performing a complete histopathologic and ultrastructural evaluation and other in-depth studies, it was found that the myocardial alteration of electrolytes and tissue fluids appeared to precede the development of the morphologic lesion. It was further found that, in addition to the heart, there were histopathologically demonstrable lesions in the bones and kidneys of these animals. Therefore, it is of interest to consider the possibility that, in the rabbit model, lesions to some affected tissues may actually precede those found in the heart. Another effort of the group that has been continuing for the past three years, studying the effects of antineoplastic agents in cancer patients, has been largely concluded. The clinical and autopsy records of the total 300 patients with neoplastic disease treated at the Clinical Center were processed using a computerized system developed in conjunction with DCRT.

It has been suggested that lysozyme, in addition to its role in host-defense against infectious diseases, plays an important role in host-defense against neoplastic disease (Osserman _et al_. Nature _243_: 331, 1973). The primary problem in defining the role of lysozyme in host-defense has been the ubiquitous nature of the enzyme. Thus this group initiated a study of the role of lysozyme in host-defense. Rabbits deficient in this enzyme were identified and the condition in these rabbits was characterized. Breeding studies suggested the trait is inherited as an autosomal recessive condition which does not result in any observed morphologic lesions. Histobacterial studies of the leukocytes from these rabbits suggested an absence of lysozyme in these cells. Evidence of an increased susceptibility to either infectious or neoplastic disease is being sought, but to date none has been discerned. The identification and characterization of lysozyme deficiency in rabbits should aid in the delineation of the yet-to-be-defined role of lysozyme.

C. Drug Interaction Unit

This Unit was created less than two years ago because of the obvious importance of studying drug interactions in a field which commonly employs multiple drug combinations. This group has expanded its capabilities so that it is now able to study not only microsomal drug metabolism but also is able to assess the effects of several soluble enzymes in animals treated with antineoplastic agents. In studies initiated last year, this group has continued, and largely concluded, work on the effects of Adriamycin, Daunomycin, and selected nitrosoureas on microsomal drug metabolism. Because the Baltimore Group has reported that there are significant differences in the distribution and metabolism of Adriamycin in patients with hepatic dysfunction, the Drug Interaction Unit studied the effects of Adriamycin and Daunomycin on a battery of microsomal parameters. These studies also are part of a program to investigate the feasibility of using such studies as early indicators of impending hepatotoxicity.
These two antibiotics, which differ only by the presence of a hydroxyl group, were found to have divergent effects on mixed function oxidase (MFO) activity. For example, Daunomycin invariably produced an increased MFO activity of about 25% for up to 17 days after a five-day course of the drug. On the other hand, Adriamycin produced significant decreases of 15 to 30% in MFO activity. These findings are of considerable importance when coupled with the clinical observations, since the possibility exists that the Adriamycin-induced inhibition of MFO activity coupled with impaired hepatic function could lead to a higher degree of toxicity then might be otherwise expected. In studying the three nitrosoureas, BCNU, CCNU, and Methyl CCNU, there were striking differences observed in the

effects of these agents, particularly after treatment with Methyl CCNU. After a four day course of treatment with this drug, the MFO activity in mice was found to be considerably enhanced for up to 17 days after treatment. The profile of induction is under further investigation, but the description of this inductive process is unlike that of any other inducing agent reported to date. This inductive effect of Methyl CCNU may explain the lesser toxicity associated with treatment with this agent as compared to BCNU and CCNU. Because the lung is the site of toxicity for some of our agents (e.g. Busulfan and Bleomycin) this Unit has continued its previous studies in the area of drug metabolism by pulmonary tissue. In an attempt to further characterize and compare drug metabolizing activity of pulmonary and hepatic tissue, the subcellular localization and activities of several transferases were determined in rabbits. The following conjugation reactions were studied: UDP-glucuronyl transferase, N-acetyl transferase, glutathione S-aryl transferase and phenolsulfotransferase. After the base line studies are completed it will be of interest to study whether such enzyme activities are altered in the presence of drug-induced pulmonary toxicity. Such studies are very important, since they point out some of the potential hazards associated with the use of such agents in patients who can little afford to be treated with combinations of drugs which might have greatly increased toxicity because of a compromised ability of the liver to catabolize other drugs which might be used in adjunct therapy. This research group has continued in the area of species differences in drug metabolism. These studies are important since one always seeks to discover "the one animal which metabolizes drugs in a manner similar to man". Often it is advantageous to discover the species which does not metabolize a drug very much since such an impaired detoxication process would exacerbate the toxicity due to the parent drug. Such manifestations of toxicity in an animal would suggest to the clinician the kinds of symptoms expected in an individual unable to detoxify by the usual metabolic processes.

D. Collaborative Efforts with other NCI Units

The Laboratory of Toxicology has continued its efforts to increase the level of interaction with other segments of the linear array. Joint efforts to improve cooperative efforts have been particularly notable with the Clinical Trials Branch. The Laboratory of Toxicology has been invited by this Branch to participate in several of the Phase I Clinical Meetings. Attendance at these meetings has been mutually beneficial. The Prime Contractor will further strengthen this interaction by setting up meetings attended by the clinicians and preclinical scientists. Cooperative efforts also have continued in the Dose Formulation area. Following the "spin-off" that resulted in a new formulation of tetrahydrocannabinol last year, we have been able to employ the vehicle emulphor in solubilizing 10 to 11 environmental agents, at least at the level of 2 mg/ml. The development of such formulations will greatly aid the environmentalist to assess the intravenous toxicity of these agents that are usually soluble only in the ppm range in the environment. Other members of the Laboratory of

Toxicology have collaborated with the Membrane Transport Section, OADET, DCT in the application of the dogfish blood-brain-barrier model to the study of about 20 antineoplastic agents. These results will be summarized elsewhere, but briefly such studies have shown that the two agents, Ellipticine and Hycanthone had brain plasma ratios of between 7 and 30, four to twenty-four hours after single injections of this material. On the strength of these findings in the dogfish, further studies were initiated to test these drugs extensively in the mouse ependymoblastoma model under a contract by the Drug Research and Development Branch.

E. Collaborative Effort with other NIH Units

The collaborative efforts of the Laboratory of Toxicology with the Division of Computer Research & Technology (DCRT) have continued. This program is involved with the development of computer-assisted methods for quantification of structure activity relationships. The primary effort of this program has been to see if there is a correlation between the mass spectrum fragmentation pattern of a drug and its pharmacologic actions. Rationale for this study was that mass spectral fragmentation patterns may be an indicator as to which portions of a drug molecule are necessary to give a certain type of biologic activity. The earlier studies which involved experimental programs to see if such a system would separate sedative from tranquilizer activity have been extended to include six other classes of drugs including some antineoplastic agents. The results of this work will be published shortly by Computers in Biology and Medicine. This project has continued to allow Laboratory of Toxicology personnel a unique opportunity to collaborate with chemists, huristics experts, computer scientists, mathematicians, and members of the Drug Research and Development Branch. This has not been a one-way street since the non-NCI members of this group have been most interested in applying their skills to the solving of very practical problems of our Division.

III. Contract Programs

A. The Prime Contract

February of this year, the Laboratory of Toxicology launched the first prime contract in the Division of Cancer Treatment. After extensive free and open competition, the Battellle Memorial Institute was the victor in this award. Although it is too early to tell about the performance of this contract, certainly the early phase of their efforts has been very promising. The entire team which has been assembled in a local office in McLean, Virginia is very enthusiastic and very competent. In a good number of the tasks not expected for completion for considerable time, they are ahead of schedule. For the first quarter of this contract year it would seem to be an advantage to have flexibility of responsiveness from this contractor since there has been a dramatic change in the kind of work requested of the Laboratory. Instead of having about ten compounds per year for protocol toxicity testing and a few for special testing, it would seem that the ratio for the early part of this year may be reversed, where only a few compounds are currently available for full testing and about seven compounds scheduled for some kind of abbreviated testing.

B. Present Contract Programs

Most of the contracts supported by the Laboratory of Toxicology for protocol toxicity studies were terminated as of March, 1974 in order to coincide with a phase-in of the prime contract effort. To date the Prime Contractor has awarded only one subcontract for the conduct of protocol studies and according to the present number of compounds (see above) this would seem to be adequate. The prime contract will start up only an appropriate number of subcontracts and hence this should be a very economic process for getting the work done. As mentioned in the early part of this summary, it is desired to get other kinds of research contracts started, especially in the mechanistic area, but such solicitations from the Laboratory will have to await additional staffing to allow for this increased supervisory effort.

Serial No. NCI-3518
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: L-Asparagine: Synthesis, Biological Role, and Hydrolysis
Streptozotocin: Alkylation of Nucleic Acids*

Previous Serial Number: Same

Principal Investigator: David A. Cooney

Other Investigators: Richard A. Rosenbluth, Hiremagalur Jayaram, James Althaus, Harry Milman

Cooperating Units: Ulrich Schaeppi, Mason Research Institute, Worcester, MA
Nathan Rakieten, South Shore Analytical and Research Laboratories, Inc., Islip, NY
John Driscoll, Division of Cancer Treatment, National Cancer Institute
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Man Years:

Total:	4
Professional:	2-1/2
Others:	1-1/2

Project Description:

Objectives:

To examine the pharmacological properties of oncolytic enzymes and to examine the mechanism of the toxicity of select nitrosoureas.

Major Findings:

The *in vitro* and *in vivo* inhibition of L-asparagine synthetase from L-asparaginase-resistant Leukemia 5178Y (L5178Y/AR) and from murine pancreas by the ketoamino acids DON, CONV and DONV was investigated, using both L-glutamine and ammonia as substrates. At a concentration of 1 mM, DON and CONV almost completely inhibited the *in vitro* utilization of L-glutamine by L-asparagine synthetase of L5178Y/AR and normal mouse pancreas, whereas DONV inhibited the enzyme from both sources by only 50%. DON, CONV and DONV did not affect the *in vitro* utilization of ammonia by L-asparagine synthetase from L5178Y/AR while DON and CONV modestly inhibited the utilization of ammonia by the pancreatic enzyme. The inhibition produced by DONV was fully and rapidly reversed by dialysis, whereas that produced by DON and DONV was essentially irreversible. DON and CONV strongly inhibited the in vitro utilization of L-glutamine by eight fetal rat liver amidotransferases examined, while DONV exerted modest inhibition of only two of these enzymes. In a survey of other enzymes using L-asparagine and L-glutamine as substrates, DONV was shown to be the best inhibitor of enzymes utilizing L-glutamine as substrate, and CONV affected both groups of enzymes to a varying degree. However, overlapping specificity was seen, as exemplified by DON, which inhibited L-asparaginase from Erwinia caratovora irreversibly. This hydrolase also was found to catalyze the decomposition of DON. DON powerfully inhibited the utilization of L-glutamine by L-asparagine synthetase of L5178Y/AR, CONV exhibited significantly less inhibition, and DONV was inert. DON and, to a lesser extent CONV, exerted strong inhibition on the in vivo utilization of both L-glutamine and ammonia by the L-asparagine synthetase of murine pancreas. DON and CONV significantly reduced the organ pool of L-asparagine in L5178Y/AR tumor and murine pancreas. Only DON enhanced the pool size of L-glutamine in both organs to a significant degree. From these findings and also from kinetic studies, it is clear that all three ketoamino acids are capable of behaving as antagonists of L-glutamine. DON is the most universally active antagonist of this amino acid, followed by CONV, and lastly by DONV. Similarly, all three agents can function as L-asparagine antagonists under appropriate conditions. Versus Leukemia 1210, DON was the most active therapeutic agent of the three drugs, whereas CONV was the most potent cytocidal agent against KB tumor cells in culture. The LD_{50}'s of the agents were dissimilar, with CONV the most toxic drug of the three following single intraperitoneal injections, and DONV the least toxic. Only DON showed evidence of prominent cumulative toxicity, thus confirming the findings of other workers. In studies with isolated cells of Leukemia 5178Y, all three agents appeared to compete both with L-asparagine and with L-glutamine for transport into the cell, but DONV competed most effectively with L-glutamine and CONV most effectively with L-asparagine. All three drugs were cleared rapidly from the plasma. None were bound to the proteins of mouse plasma. By amino acid analysis, DONV was identified at a concentration of 1 mM in tumor and pancreas 3 to 6 minutes after the intraperitoneal injection of a dose of 100 mg/kg. Although the metabolism of all three of these ketoamino acids was found to be minor in degree, evidence is presented that they can undergo transamination. In the case of DONV, some conversion to CO_2 by isolated tumor cells was also observed. It is concluded that DON is the most "L-glutamine-like" and L-DONV the most "L-asparagine-like". CONV has important attributes of both amino acids.

Studies with NSC-163,501 (5-Isoxazole-acetic acid, α-amino-3-chloro-4,5-dihydro-)

In view of its structure, this new oncolytic antibiotic from Upjohn might be supposed to be an antagonist of L-glutamine. This anticipation has been verified in several systems. The results of our studies on the agent and a congeneric antibiotic can be epitomized as follows:

NSC-163,501 (5-isoxazoleacetic acid, α-amino-3-chloro-4,5-dihydro-) and its hydroxy analogue, NSC-176,324 (5-Isoxazoleacetic acid, α-amino-3-chloro-4,5-dihydro-4-hydroxy-), are powerful inhibitors of L-asparagine synthetase both in vitro and in vivo when L-glutamine is utilized as an amide-donor. The type of inhibition produced in vitro is concluded to be classically competitive on the basis of graphical analysis, but to be at least partially irreversible on the basis of dialysis experiments. L-Glutamine, in large molar excess to the inhibitor, can partially antagonize i its inhibition. Attempts to demonstrate alteration of the inhibitor molecule in the act of catalysis were unsuccessful.

Studies with Ethacrynic Acid

During the past year, studies on the nature of the inhibition exerted by ethacrynic acid versus tumoral and pancreatic L-asparagine synthetase have been concluded. The following statements represent the results of the studies:

Ethacrynic acid, a clinically useful diuretic, has been shown to inhibit L-asparagine synthetase from Leukemia 5178Y/AR in vitro. This inhibition is thought to involve the formation of an adduct between ethacrynic acid and sulfhydryl functions on the enzyme; the adduct is not readily reversible even when another thiol, such as dithiothreitol, is used to displace the acid. When a series of analogs of ethacrynic acid were examined as inhibitors of L-asparagine synthetase, only one proved to be superior to the title compound: it was 2-(1-carboxymethoxy-4-chloro-4-naphthyl)-3, 6-diethyl-6-(1-carboxymethyoxy-4-chloro-2-naphthyl)-5,6-dihydro-[4H2-pyran]. There was no correlation between the diuretic or natriuretic potency of the series of acids examined and their ability to inhibit L-asparagine synthetase. There was no correlation between the ability of these agents to inhibit L-asparaginase from Dasyprocta aguti (another "sulfhydryl" enzyme), and L-asparagine synthetase. In every case, ethacrynic acid and its analogs interrupted the utilization of ammonia by L-asparagine synthetase to a greater degree than the utilization of L-glutamine. In vivo, the inhibition of L-asparagine synthetase in subcutaneous nodules of Leukemia 5178Y/AR by ethacrynic acid was feeble and transitory.

Studies with Mucobromic and Mucochloric Acids

Dr. John Driscoll of NCI selected mucochloric and mucobromic acids as ptoential inhibitors of L-asparagine synthetase on the basis of their structural resemblance to L-aspartic acid. The results of our studies with these halogenated acids can be summarized as follows:

The halogenated organic compounds, mucochloric acid and mucobromic acid, are powerful inhibitors of L-asparagine synthetase from Leukemia 5178Y and from murine pancreas. Two nitrogen donors, L-glutamine and ammonia, can be used by these enzymes; at a concentration of 1 mM, mucochloric and mucobromic acids preferentially inhibit the utilization of ammonia as opposed to L-glutamine. Using the tumoral enzyme, kinetic analysis revealed that mucochloric and mucobromic acids produced inhibition which was apparently non-competitive with ammonia and L-aspartate, but of mixed type with L-glutamine. Dithiothreitol efficaciously blocked the inhibition exerted by both mucochloric and mucobromic acids; dialysis failed to reverse established inhibition. These findings suggest that the drug operates by covalent attachment to crucial sulfhydryl functions on the enzyme. *In vivo*, mucochloric acid proved to be a moderately powerful but transitory inhibitor of tumoral and pancreatic L-asparagine synthetase. Mucobromic acid was devoid of therapeutic activity against Leukemia 5178Y.

Studies with aminomalonamide

In preceeding annual reports, studies on the inhibition of L-asparagine synthetase by aminomalonic acid and its derivatives were discussed in detail. During the past year the additional biochemical pharmacologic studies with this family of dicarboxylic amino acids have been undertaken. The affinity (K_m) of renal and hepatic peptidases for the diamide of aminomalonic acid has been measured. The enzyme from both sources deamidates aminomalonamide maximally at 0.02M with 1/2 V_{max} at 0.003M. The same constants were found to apply to the crystalline enzyme, leucine amino peptidase. The feeding of aminomalonamide to mice as 2% of the diet produces severe overt neurological derangements and striking neuronolysis of the cerebral cortex after 3-5 days. Therefore, plans are underway to examine the therapeutic activity of the agent in the transplantable murine ependymoblastoma (in conjuction with Drs. John Venditti and Ruth Geran).

Analytical Studies:

Development of a new technique for measuring L-asparagine synthetase

As presently carried out, the analysis of L-asparagine synthetase requires about 8 hours to complete. Under ordinary circumstances this span offers no problems. However, when purification of the enzyme, a notoriously labile protein, is being attempted there is real need for speed in order that subsequent steps can be carried out before denaturation supervenes. For this reason we have been examining the possibility of accelerated techniques. One of these utilizes elevated temperatures to decarboxylate the [^{14}C]-L-aspartic acid not consumed in the synthesis of L-asparagine. We have observed that L-glutamate oxaloacetate transaminase (the reagent enzyme utilized to convert the L-aspartate to oxaloacetate which is, in turn, quantitatively β-decarboxylated by zinc ions) is stable at 56° for at least 2 hours. The same is true of L-asparaginase, the second reagent-enzyme used. Moreover, oxaloacetate is β-decarboxylated about 3-5 times more rapidly at 56° than at 37° and CO_2 is expelled very efficiently at the higher temperature. Because of these features, it has been possible to reduce the analysis to 2 hours.

In order to gain some insight into the mechanism of the reaction catalyzed by L-asparagine synthetase it would be advisable to study the partial and reverse reactions catalyzed by the enzyme. Many synthetases will exchange ^{32}phosphate or ^{32}pyrophosphate with ATP to yield phosphate or pyrophosphate and ^{32}P-ATP. If ATP affixes first to the enzyme, it is likely that such an exchange will transpire in the absence of any other substrates. Chou and Handschumacher proposed that L-glutamine or ammonia were the first reactants in the reaction catalyzed by L-asparagine synthetase from the 6C3HED lymphoma (therefore, that the enzyme was aminated or amidated as a first step), that ATP was utilized next, and then L-aspartate entered and was amidated. This postulate would seem to require ATP-^{32}pyrophosphate exchange in the presence of L-glutamine or NH_3 alone. In order to examine the point experimentally, we have developed a facile analytical technique for separating ATP from pyrophosphate, based on the differential solubility of these two compounds in 80% ethanol. Advantage has been taken of the finding that pyrophosphate is virtually insoluble in 80% ethanol, whereas ATP is fully soluble. Since ATP will exist as the magnesium-chelate in most synthetase reaction mixtures, and since ATP-Mg is insoluble in 80% ethanol, the technique uses a saturated solution of sodium citrate to scavenge magnesium ions. Carrier cold pyrophosphate is also utilized to aid in the precipitation of the pyrophosphate pellet. In practice, the enzyme is incubated with ^{32}pyrophosphate and one of the substrates in a volume of 10 µL. After an appropriate time, 50 µL of a saturated solution of sodium citrate in 0.2M sodium pyrophosphate, pH 9.0, is added and followed by 1 ml of 80% ethanol. The vessels are spun at 12,000g for 3 minutes and 100 µL of the ethanoloic supernatant counted in a scintillation spectrometer.

Studies with New Oncolytic Enzymes

During the past year, reports have appeared describing the antitumor activity of several amino acid-degrading enzymes. We have had the opportunity of studying one of these: L-phenylalanine-ammonia lyase (PAL), a protein catalyzing the conversion of L-phenylalanine to cinnamic acid and ammonia or of L-tyrosine to coumaric acid and ammonia. Dr. C. W. Abel of the University of Texas, Galveston, Texas, purified and provided this enzyme to Dr. Florence White of NCI for therapeutic testing against Leukemia 1210 and Leukemia 5178Y/AS. This laboratory examined the toxic properties of the preparation. Unfortunately, at doses calculated to be therapeutic, the enzyme killed 100% of the recipients. This effect was felt to be due to endotoxin, in the light of the time-course of the deaths and the necrotic changes which were detectable grossly and histopathologically in the livers of animals succumbing to the preparation. Repurification is underway in Dr. Abel's laboratory.

Studies with L-Glutaminase from *Aerobacter aerogenes*

At the University of Delaware, Dr. John Wriston has partially purified a new L-glutaminase from *Aerobacter aerogenes* that is active at physiological pH. This enzyme has been examined here for its acute toxicity in mice in view of the strong propensity of analogous hydrolases to become contaminated with, or to retain endotoxin. Doses of 1 to 100 I.U./kg proved to be well tolerated. The $t_{1/2}$ approximated 3 hours. Twenty-four hours after the intravenous injection of 50 I.U./kg, plasmatic L-glutamine was at a normal level.

The Effects of Streptozotocin (Glucopyranose, 2-deoxy-2-(3-methylnitrosoureido)-D-) (NSC-85998) on Mammalian Nucleic Acids

Streptozotocin is a nitrosourea antibiotic with significant oncolytic activity. Its glucosamine function has been postulated as the basis for its affinity for pancreatic islet beta cells. In fact, Streptozotocin is presently the drug of choice for metastatic islet cell tumors of the pancreas in man. In addition to its oncolytic activity, Streptozotocin has been shown to destroy normal beta cells of the pancreas, resulting in a diabetic syndrome in many species but not in man. Nicotinamide effectively blocks this action. Finally, Streptozotocin administered to Holtzman rats pretreated with nicotinamide, has been shown to cause nesidioblastomas (islet cell tumors of the pancreas) within 300-500 days. The objectives of the present study were: (1) To investigate the effects of Streptozotocin on nucleic acids in mice and rats, and more specificially to search for evidence of methylation of purine residues; (2) To quantitate the degree of methylation of purine residues in normal tissues, in a tumor known to be sensitive to Streptozotocin (L1210), and in a tumor known to be resistant to Streptozotocin (Lewis Lung); (3) To quantitate methylation in Streptozotocin-induced rat nesidioblastomas as well as in normal rat pancreas and to compare this with the degree of methylation produced by an equivalent dose of N-methyl-nitrosourea, and (4) To study the effects of alkylation on thymidine incorporation into various nucleic acids as evidence of altered nucleic acid function. In mice treated with labeled Streptozotocin, 7-methyl guanine was readily detectable. Approximately 0.3% of guanyl residues were alkylated in both livers and kidneys of mice, a degree comparable to that produced by other nitrosoureas and nitrosamines. No other methylated products could be detected, with the exception of an early appearing, acidic, volatile substance that may be methanol or methylamine. 7-Methyl guanine was also detectable in the nucleic acids of rat nesidioblastomas, although the degree of methylation is yet to be determined. Streptozotocin alkylated calf thymus DNA and yeast RNA in vitro, yielding 7-methyl guanine in each case. The kinetics of methylation were examined and the results indicated linear increase in methylation up to 8 hours of incubation at 37°. The degree of alkylation levelled off thereafter. The degree of methylation also varied directly with the concentration of nucleic acid in the incubation medium up to a concentration of 100 mg/ml, at which time it levelled off. The incorporation of labelled thymidine into DNA of mouse liver and L1210 tumor is significantly depressed by Streptozotocin; however this depression is far less than that produced by the parent drug, N-methyl-nitrosourea.

Significance to Biomedical Research and the Program of the Institute:

To date, the number of enzymes that have oncolytic activity is small, and confined mainly to hydrolases capable of eradicating essential or quasi-essential amino acids. Nevertheless, it appears warranted to generalize that such enzymes are powerful, and on occasion, selective tools for attacking cancer. The present series of studies seeks to uncover small molecules which will broaden or enlarge the therapeutic scope of such exogenous enzymes by inhibiting the induction or function of endogenous synthetases responsible for resistance. Studies on the alkylation of DNA by Streptozotocin suggest a mechanism for the oncogenicity of this drug.

Proposed Course:

Systematic screening for inhibitors of L-aspargagine synthetase will continue. Studies on new oncolytic enzymes will continue. It is hoped that non-denatured extracts of cultures and plants will begin to be examined in the anticipation of the discovery of marcromolecular oncolytics in these sources. In the studies of nitrosourea toxicity, it is proposed to compare methylation produced by Streptozotocin in L1210 vs. Lewis Lung, as discussed above. The degree of methylation produced by Streptozotocin compared with N-methyl nitrosourea in the nucleic acids of nesidioblastomas and normal rat pancreas will also be examined. Studies of the kinetics of thymidine incorporation into the DNA of animals treated with Streptozotocin and with N-methyl nitrosourea will be completed as will a comparison of the inhibition of thymidine incorporation into the DNA of Lewis Lung with that of L1210.

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[*] This paper was previously entitled, L-Asparagine: Synthesis, Biological Role, and Hydrolysis

Serial No. NCI-6110
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Pathology of Drug-Induced Alterations in Animals and Man

Previous Serial No. Same

Principal Investigator: David M. Young

Other Investigators: Harry M. Olson, David J. Prieur, David P. Houchens, Louis B. Thomas, Arnold W. Pratt, and Elton R. Homan

Cooperating Units: Laboratory of Pathology, DCBD, NCI
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Immunotherapy and Immune Response Modifiers Section, DCT, NCI

Man Years:

Total:	2-1/2
Professional:	1-1/2
Other:	1

Objectives:

1. To contribute to a further understanding of the pathogenesis of L-asparaginase-induced hypocalcemia in rabbits.
2. To define the clinicopathologic effects of Adriamycin in rabbits.
3. To characterize the transplantable VX_2 carcinoma in rabbits as a model for:
   a. hypercalcemia of malignancy
   b. therapy with antineoplastic agents (i.e. Adriamycin, Mithramycin)
4. To define the pathologic effects of an intracerebral murine glioma model.
5. To systematically evaluate and record reports of adverse effects of antineoplastic agents in cancer patients on a computerized basis and correlate these findings with those recorded in test animals.

Major Findings:

1. L-Asparaginase-induded hypocalcemia in rabbits

The intravenous administration of Escherichia coli L-asparaginase (Asnase) to rabbits at a dose schedule of 1000 IU per kg. per day for 3 days resulted in an incidence of hypocalcemia of 100 percent and tetany of 70 percent. The majority of the rabbits had hyperphosphatemia, hypomagnesemia, hyperkalemia, and azotemia. These alterations correlated well with histopathologic and ultrastructural findings of degenerating chief cells, degranulation, autophagy, and deficiency of subcellular organelles associated with protein synthesis in parathyroid glands.

Additional studies were designed to contribute to a further understanding of the Asnase-induced parathyropathy. Separate groups of rabbits under went surgical thyroparathyroidectomy, bilateral ureterectomy, treatment with Erwinia carotovora-Asnase, parathyroid extract and calcium gluceptate. The results of these investigations support the hypothesis that the parathyroid alteration associated with Asnase administration is related to the cause, rather than the effect, of the hypocalcemia. In addition, the results indicate that neither endotoxin nor the uremic effects of Asnase are etiologic in the development of the experimentally induced hypocalcemia and tetany. The rabbit-Asnase system is presented as an experimental animal model of drug-induced hypoparathyroidism characterized by drug-endocrine cell interactions somewhat analogous to the lethal selectivity of alloxan for pancreatic β-cells in the production of experimental diabetes.

2. To elucidate the clinicopathologic effects of Adriamycin in the Rabbit
Preliminary studies of the cardiotoxic effects of Adriamycin revealed a striking difference in the incidence of cardiomyopathy between conventional and specific pathogen free (SPF) (minimal disease) rabbits. A significant number of conventional rabbits died with intercurrent infections prior to or at the onset of congestive heart failure. Therefore further investigations were performed in SPF rabbits housed in a room isolated from other animals. The intravenous administration of Adriamycin to SPF rabbits at a dose schedule of 0.7 mg/kg (7.7 mg/M^2) 3x/week resulted in a 100% incidence of a fatal cardiotoxic syndrome at total cummulative doses ranging from 254-416 mg/M^2. The cardiotoxic syndrome was characterized by generalized subcutaneous edema, ascites, bilateral pleural effusions and morphologic alterations in the myocardium. A study of rabbits sacrificed at total cumulative doses of 200, 300, and 400 mg/M^2 revealed that only rabbits at the 400 mg/M^2 level had evidence of cardiomyopathy. These results indicate that the rabbit is more sensitive to Adriamycin than man. The critical cardiomyopathic dose for rabbits is equal to or greater than 250 mg/M^2, whereas for cancer patients it is equal to or greater than 550 mg/M^2.
Histopathologic and ultrastructural evaluation of myocardium from Adriamycin treated rabbits with the cardiotoxic syndrome revealed characteristic lesions of edema, fibrosis, myocytolysis, vacuolization of myocytes, loss of myofibers, distentions of sarcoplasmic reticulum and multilaminated whorls (myelin-like figures) encircling degenerating mitochondria. These findings were correlated with water and mineral analyses of myocardium from treated and control rabbits. Rabbits with cardiomyopathy had significantly increased myocardial fluid content, and increases in calcium (202%) and sodium (130%) concentrations in heart muscle compared to controls. In addition, cardiomyopathic rabbits had decreases of myocardial magnesium (70%) and potassium (74%) compared to controls. The aforementioned alterations did occur, albeit to a lesser degree, in Adriamycin treated rabbits not developing the cardiotoxic syndrome. Therefore the myocardial alterations of electrolytes and tissue fluid content appears to precede, and are considered to be of etiologic importance in, the development of the cardiomyopathy. Further studies are in progress to test the hypothesis that Adriamycin administration results in altered myocardial membrane permeability.

An abnormal subcellular translocation of myocardial electrolytes and tissue fluids would interfere with contraction-relaxation coupling and result in irreversible degeneration of myocytes.

Gross and microscopic evaluation of tissues collected at necropsy from Adriamycin treated rabbits revealed significant alterations in bones and kidneys compared to controls. The occurrence of bone and kidney lesions appeared to be dose and time dependent and preceded the development of cardiotoxicity. Further studies are in progress to better define the pathogenesis of the Adriamycin-induced glomerulonephrosis in rabbits.

Physicochemical and morphologic changes were evident in bones from Adriamycin treated rabbits. Evaluation of bones selected from axial and appendicular portions of the skeleton revealed significant increases in wet weight, decreases in density and increases in the concentration of calcium and phosphorus content of bone ash. The more significant lesions occurred at metaphyseal and epiphyseal regions of long bones and consisted of dysplasia of the cartilage plate, loss of primary and secondary spongiosa, increased content of mesenchymal-type tissue with depletion of osteoblasts and osteoclasts. Preliminary incubation studies indicate that Adriamycin binds readily to fresh, but not decalcified, cortical bone powder. The effects of Adriamycin on bone may be due to an inhibitory action on osteoprogenitor cells and/or formation of Adriamycin-calcium-phosphate complexes that interfere with bone turnover.

3. Transplantable VX_2 Carcinoma in Rabbits as a Model for (a) hypercalcemia of malignancy and (b) therapy with antineoplastic agents (i.e. Adriamycin and Mithramycin)

Tumor stock of the VX_2 carcinoma was obtained from Arthur D. Little, Inc. and transplanted, using standard procedures, every 14-21 days into the quadriceps muscles of 8 week old male New Zealand SPF rabbits. Initial studies were designed to characterize the pathologic effects of VX_2 carcinoma in rabbits. Histopathologic evaluation of tissues collected at necropsy revealed that the VX_2 carcinoma metastasized to regional lymph nodes (inguinal and sublumbar) and lungs. Analyses of serum samples revealed a significant hypercalcemia in tumor bearing rabbits. Previous reports indicate that the hypercalcemia in rabbits may be related to release by tumor cells of some as yet unknown factor(s) which have a calcium mobilizing effect on bone.

Currently 20 rabbits have been innoculated with VX_2 carcinoma cells. Twelve days post-innoculation 10 rabbits were started on Adriamycin treatment at a schedule of 6.3 mg/kg (69 mg/M^2) every third week and 10 were injected with comparable volumes of saline. Future studies are designed to determine the therapeutic effects of Adriamycin and Mithramycin utilized singly against the VX_2 carcinoma. Additional investigations are designed to characterize the pathogenesis of the tumor-mediated hypercalcemia.

4. Pathologic effects of an intracerebral murine glioma model

Mice were injected intracerebrally with ependymoblastoma glioma-26 cells harvested from monolayer cultures. Control mice were injected in a similar manner with saline. Mice were sacrificed periodically over a 28 day period and necropsies performed. Representative coronal sections of entire skull with brain intact were made passing through the injection site in the cranium.

Comparative studies of in vivo and in vitro tumor growth revealed that the monolayer cells had a higher mitotic index and more pronounced nuclear pleomorphism than intracerebral tumors. Histopathologically, transplanted tumors from mice, were highly cellular with scant stroma and no indication of inflammatory or fibrous reaction. Focal areas of hemorrhage and necrosis were occasionally evident. Tumor cells maintained ependymal characteristics with presence of cilia and blepharoplasts. The neoplasm appeared to grow mainly by expansion with displacement of brain tissue although several areas of infiltrative growths were evident in the calvarium, subdural space and ventricular system of the brain.
The results of the present investigation indicate that the glioma-26 ependymoblastoma in mice may be an ideal malignant brain tumor model which can be maintained in vitro and utilized for immuno- and chemo-therapy studies.

5. Adverse Effects of Antineoplastic agents in cancer patients
A specialized system of information storage and retrieval was designed and implemented in collaboration with the Division of Computer Research and Technology. The system was designed to process material derived from clinical and autopsy records on cancer patients treated at or referred to the NIH Clinical Center. The information processing system employs natural language and the Systematized Nomenclature of Pathology (SNOP). The major objective of the current studies is to determine the qualitative and quantitative aspects of antineoplastic therapy-induced adverse effects present in cancer patients at the time of autopsy. Currently clinical and autopsy records on a total of 300 cancer patients have been processed via the computerized system. Septicemia was confirmed at the time of death in 154 of the 300 patients. A total of 22 patients were considered clinically to be in remission at the time of death and died of secondary causes. Secondary neoplastic processes, that is those not associated with primary admitting diagnosis, were evident in 88 patients. The 10 most frequently utilized antineoplastic agents were matched against the 23 most common pathologic changes considered not to be associated directly with the neoplastic process. Lesions most frequently associated with antineoplastic therapy included aspermatogenesis, hemosiderosis, gastrointestinal tract ulceration, pneumonia, pulmonary fibrosis, bone marrow hypoplasia, hepatic fibrosis, abscesses, alopecia, congestive heart failure, renal tubular necrosis and esophagitis. Studies are in progress to determine the percent incidence of drug-related lesions occurring in patients receiving one particular anticancer agent in comparison to the incidence of specific lesions in patients not receiving the agent. The anticancer agent-associated lesions in patients will be compared with those reported previously in preclinical toxicologic studies in dogs and monkeys. These studies will contribute to a better understanding of comparative and predictive toxicology of antineoplastic agents.

6. Quantitative analysis of preclinical toxicologic effects of antitumor chemotherapeutic agents
A major portion of the preclinical evaluation of potential antitumor drugs is directed towards examination of toxic effects. In order to assure that these efforts are effectively directed towards satisfying the critical needs of the chemotherapy program, continuing appraisal of the toxicologic

protocols is essential. The immediate purpose of the present effort is to determine the quantitative relationships of toxic effects observed in single dose and multiple dose studies in order to determine the specific contributions to the total body of information on a drug contributed by the two types of study.

These studies are still in progress and only partial results are available at this time. Thirty-four dog studies have been identified to date as constituting pairs of single and daily dosing regimens suitable for comparing toxic effects. Clinical, hematologic, chemical, and pathologic alterations were scored under the appropriate organ systems. Organ systems catalogued represent those most frequently encountered in the pre-clinical studies of this laboratory: bone marrow, lymphoid, gastrointestinal, hepatobiliary, renal, reproductive and endocrine, neuromuscular, respiratory, cardiovascular and integumentary. Categories for injection site effects and for miscellaneous infrequent effects completed the series. The specific categories of organ systems are arbitrary and represent a compromise between adequate detail and convenience.

The first phase of the analysis of data was to tabulate the number of animals demonstrating specific organ system effects at each dose level. These data were examined to determine which organ systems were affected by a given drug and regimen.

Final tabulation of data in this phase was in a table for each organ system in which each regimen was assigned to one of four cells depending on the occurrence or non-occurrence of toxicity in single and multiple dosage regimens respectively ("Both", "Neither", "Single Dose Only", or "Multiple Dose Only").

One way of evaluating regimen effects is to measure the common occurrence of both the presence and absence of toxic effects. Organ systems scoring high in inter-regimen consistency of toxic effects (0.9 or greater) were hepatobiliary, cardiovascular, and integumentrary. Gastrointestinal and neuromuscular effects were least consistent.

A somewhat different approach involves determining the incidence ratio (the sum of "Multiple Only" plus "Both" divided by the sum of "Single Only" plus "Both"). In this approach, the outstanding parameter was injection site. This reflects the greater probability of extravasation or local inflammatory response with an increasing number of injections.

Another aspect under consideration is the association between the increment in the incidence of toxic effects and dosage increment. One can determine by calculating and tabulating such data whether some organ systems are most often associated with lethal dosage increments. Preliminary results suggest that, in single dose dog studies, those systems affected most frequently by lethal doses are the respiratory, hepatobiliary, and lymphatic organs. This effect on lymphopoiesis is conspicuously different from hematopoietic effects which appear more often at sublethal doses. A possibly significant difference between single and multiple dose regimens is reflected in a greater association between gastrointestinal effects and lethality in multiple dose regimens. The findings may be summarized as indicating that single and multiple dose regimens tend to produce similar patterns of toxicity, although these patterns appear to be more adequately defined in multiple dose studies.

Finally, this review of toxicologic data generated by the NCI Chemotherapy Program from 1955 to the present allows a perspective. The detail and quality of data and reporting have increased progressively over the years. The earlier data tend to be anecdotal and fragmentary. Many animals were given escalated doses, thus denying the availability of pathologic data and often obscuring delayed toxicity and recovery processes.

As the Program needs evolved, they are reflected in trends in approach and reporting. Most recent studies have greater consistency in scope and technical adequacy as well as greater detail. These are obtained, however, at greater direct financial cost and involve a burden of probably irrelevant detail.

A detailed analysis of the raw data collected in the preclinical toxicology studies would undoubtedly permit significant savings and improvements in the efficiency of the program. Such an analysis would require resources, methodology, and personnel not available at the present time. The potential rewards, however, indicate the desirability of such an undertaking.

Significance to Biomedical Research and the Program of the Institute:

The need for a better understanding of the pathogenesis of anticancer drug-induced lesions in experimental and clinical studies can not be overemphasized. The elucidation of the mechanism of Adriamycin-induced cardiotoxicity may lead to establishment of a therapeutic or prophylactic procedure to negate the cardiac failure syndrome. Results of current studies indicate that agents designed to maintain myocardial membrane permeability and/or block accumulations of myocardial calcium may alleviate the cardiotoxic activity of the drug.

The characterization of an animal model that develops an Adriamycin-induced cardiomyopathy with congestive heart failure will provide a system for screening Adriamycin analogues. The rabbit model appears to be ideal because (1) small amounts of drug can be used, (2) multiple intravenous injections can be made, (3) a reproducibly high incidence of cardiomyopathy is produced and (4) preliminary analytical data indicates that the route of metabolism of anthraquinone antibiotics is similar in the rabbit and man.

Hypercalcemia of malignancy is a well known, frequently life-threatening phenomenon. It occurs in about 40% of all cases of breast cancer. The pathogenesis of the hypercalcemic crisis occurring in cancer patients is poorly understood. The VX_2 carcioma in the rabbit appears to be an excellent model to study the mechansims involved in the genesis of the hypercalcemia of malignancy and to test therapeutic measures to control the apparent excessive mobilization of calcium stores.

The characterization of the murine brian tumor glioma-26 will provide an animal model for experimental chemo- and immunotherapeutic studies. A number of brain-tumor animal models have been proposed for chemotherapeutic studies, but few have the advantage of maintaining a stock of neoplastic cells in parallel in monolayer cultures. Harvesting large numbers of cell cultures will allow for treatment of tumor bearing mice with pre-treated cells to alter immunocompetence.

A systematic evaluation of clinical and autopsy records from cancer patients for anticancer therapy-induced adverse effects is of paramount importance in evaluating current preclinical toxicologic studies. Comparative computerized evaluations of specific drugs used in test animals and patients will provide an index into the predictiveness of current protocol studies. In addition, a computerized program to evaluate therapeutic procedures and pathologic alterations in cancer patients would be beneficial to identify specific, as yet undefined, drug associated lesions.

Proposed Course:

The pathogenesis of L-asparaginase-induced hypocalcemia in rabbits has been clearly defined and has reached a logical conclusion.
The computerization of clinicopathologic data from cancer patients and dogs and monkeys used in preclinical toxicologic studies will be handled by the Prime Contractor in the near future.
Intramural research activity will be continued and expanded under the following specific objectives: a) to define methods to inhibit myocardial calcium uptake in rabbits treated with Adriamycin and/or block the development of the degenerative cardiomyopathy; b) to better define the interactions of Adriamycin and altered bone metabolism; c) to characterize the factor(s) responsible for hypercalcemia associated with the VX_2 carcinoma in the rabbit and to determine their therapeutic sensitivity.

Publications:

1. Young, D.M., Olson, H.M., Prieur, D.J., Cooney, D.A. and Reagan, R.L.: Clinicopathologic and ultrastructural studies of L-asparaginase induced hypocalcemia in rabbits: An experimental animal model of acute hypoparathyroidism. Lab. Invest. 29: 374-386, 1973.

2. Prieur, D.J., Young, D.M., Reagan, R.L. and Olson, H.M.: Induction of anomalous lysosomes in the renal papillae of beige mice by experimental potassium deficiency. Proc. Soc. Exp. Biol. Med. 144: 683-687, 1973.

3. Black, H.E., Capen, C.C., and Young, D.M.: Ultimobranchial thyroid neoplasms in bulls: A syndrome resembling medullary thyroid carcinoma in man. Cancer 32: 865-878, 1973.

4. Homan, E.R. and Dixon, R.L.: Prediction from laboratory animals to man - quantitative relationships. Proc. Fifth Internat. Congr. on Pharmacol. 3: 289-303, 1973.

Serial No. NCI-6113
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Program
July 1, 1973 through June 30, 1974

Project Title: The Detection, Quantitation, Evaluation and Prediction of the Pathologic Effects of Antineoplastic Agents, Especially on the Hematic System, and Studies of Other Conditions of Host-Defense Failure

Previous Serial Number: Same

Principal Investigator: David J. Prieur

Other Investigators: David M. Young, Harry M. Olson and Reginald L. Reagan

Cooperating Units: Division of Cancer Treatment

Man Years:
Total: 1
Professional: 0.5
Others: 0.5

Project Description:

During the past year studies were performed in three main areas. First a study of the role of the enzyme, lysozyme, in host-defense was begun. Secondly, studies were undertaken to determine the relationship between partially albino white-tailed rats (Mystromys albicaudatus) and the Chediak-Higashi syndrome, a host-defense failure disease. Thirdly, studies were undertaken to determine the relationship between potassium deficiency and the Chediak-Higashi syndrome.

Major Findings:

Rabbits deficient in the enzyme lysozyme were identified and procured, and the condition in these rabbits was characterized and compared to similar conditions in other species. The rabbits had no morphologic lesions at either the light or electron microscopic level in any tissues examined. Breeding studies performed suggest the trait is inherited as an autosomal recessive condition. Histobacterial studies of the leukocytes from these rabbits suggested an absence of lysozyme in these cells. However, further studies of a large number of tissues from lysozyme-deficient and control rabbits demonstrated that lysozyme was present in these rabbits but at levels less than 1% of normal in most tissues. No inhibitors of lysozyme activity could be found in the tissues of the lysozyme-deficient rabbits. In vitro studies of the lytic activity of leukocytes from control and lysozyme-deficient rabbits against the bacteria, Micrococcus lysodeikticus, revealed an absence of such activity in the lysozyme-deficient leukocytes at 40 minutes. No clinical evidence of an increase in susceptibility to either infectious or neoplastic disease has been discerned.

It was determined that the partially albinotic condition in Mystromys albicaudatus was not homologous with the Chediak-Higashi syndrome.

It was determined that normal mice fed a potassium deficient diet develop a large number of large lysosomes in the cells of the renal papillae. It was further determined that mice with the Chediak-Higashi syndrome develop a smaller number of much larger lysosomes under the same conditions suggesting that fusion of granules plays a role in the development of the enlarged lysosomes in the Chediak-Higashi syndrome.

Significance to Biomedical Research and the Program of the Institute:

It has been suggested that lysozyme, in addition to a role in host-defense against infectious disease, plays an important role in host-defense against neoplastic disease (Osserman *et al*. Nature 243: 331, 1973). The primary problem in defining the role of lysozyme in host-defense has been the ubiquity of the enzyme; i.e., control cells without lysozyme are not available. The identification and characterization of lysozyme deficiency in rabbits may thus aid in the delineation of the role of lysozyme.

The Chediak-Higashi syndrome is a condition reported in man and several other species in which there is an increased susceptibility to both infectious and neoplastic disease. In addition persons and animals with this condition manifest a partial albinism and their cells contain enlarged lysosomes. When it was reported that rats with a partial albinism might represent an additional model of this syndrome it was important to determine whether the condition in the rat was homologous with that of the Chediak-Higashi syndrome of other species. We have established that the conditions are not homologous.

Since both the Chediak-Higashi syndrome and experimental potassium deficiency are manifested by apparent alterations in lysosomes and lysosomal membranes it was thought that there might be a relationship between the two conditions. When it was determined that the presence of the gene for Chediak-Higashi syndrome in mice accentuated the fusion of granules forming lysosomes in the renal papillae it raised the possibility that the basic mechanisms of the two conditions may be similar.

Proposed Course:

The areas of work enumerated above will be continued during the coming fiscal year.

Publications:

1. Prieur, D.J., Young, D.M., Reagan, R.L., and Olson, H.M.: Induction of anomalous lysosomes in the renal papillae of beige mice by experimental potassium deficiency. *Proc. Soc. Exp. Biol. Med*. 144: 683-687, 1973.

2. Guarino, A.M. and Prieur, D.J.: Increasing the reliability of extrapolation of preclinical toxicologic data of antineoplastic agents. In *Pharmacological Basis of Cancer Chemotherapy: Toxicology of Anticancer Drugs*. Williams & Wilkins Co., Baltimore, MD (in press) 1974.

3. Reagan, R.L., Weinstein, A.J., Green, C.A., and Adamson, R.H.: Virus-like particles in lymph nodes from patients with Hodgkins disease. Soc. Exp. Biol. Med. (in press) 1974.

Serial No. NCI-6122
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Program
July 1, 1973 through June 30, 1974

Project Title: Drug interactions and other factors influencing the physiological disposition and toxicity of drugs.

Principal Investigators: T. E. Gram, C. L. Litterst and A. M. Guarino

Other Investigators: E. R. Homan, R. L. Reagan, A. Leroy, D. Prieur

Cooperating Units: Biomedical Engineering and Instrumentation Branch, Division of Research Services

Man Years:
Total: 3.2
Professional: 2.2
Other: 1.0

Project Description:

Objectives:

The interaction between a drug and tissue receptors which ultimately produces a pharmacologic or toxicologic response is the product of a large number of attenuating factors. For example, the distribution of drugs within the body depends upon a balance between relative organ perfusion, the affinity of the drug for binding sites within different organs, the extent of binding of the drug to plasma proteins, and the rate of its excretion into the urine and bile. Moreover, the metabolic clearance of drugs from the body depends upon their relative rates of biotransformation to products which are more polar and more rapidly excreted. Thus, absorption, distribution, binding, metabolism, and excretion tend to dictate the steady-state plasma drug concentration under specified conditions and thus determine the pharmacologic or toxicologic response. Since alterations in these factors are known to either diminish or intensify biologic responses, the need to assess these parameters quantitatively is of obvious therapeutic significance. Thus, studies have been initiated in the Drug Interactions Unit to investigate and appraise these parameters regarding drugs in general, and oncolytic agents in particular.

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

A. The influence of selected antineoplastic agents on drug metabolism by enzymes in hepatic microsomes. Single doses of a number of antineoplastic drugs produce a roughly dose-dependent inhibition of microsomal drug metabolism in rats and mice. With the exception of the nitrosoureas whose effects persist for at least five weeks, the enzyme inhibition is most profound during the first 3-4 days and enzyme activities generally return to control levels in 1 to 2 weeks. Studies with other drugs such as phenobarbital have shown that daily administration for 3 to 7 days produces a profound stimulation of microsomal enzyme activity with a concomitant reduction in the duration or intensity of action of many other drugs. It is of interest that many such enzyme "inducers" exhibit biphasic effects on microsomal enzymes, i.e., an initial impairment or inhibition followed later by stimulation. Although single doses of many carcinolytic agents had been shown to inhibit microsomal enzymes, the possibility that multiple doses might be associated with enzyme induction has not been explored adequately. Our plan was therefore to administer daily therapeutic doses of several antineoplastic agents for 5 days and to examine microsomal enzyme activities at several time points afterward (1, 5, 10 and 14 days). As an extension of work begun last year, we have examined the effects of Adriamycin, Daunomycin and selected nitrosoureas on microsomal drug metabolism.

1. Adriamycin and Daunomycin, two antineoplastic drugs differing structurally only in the presence of a hydroxyl group, were tested for their effects on liver microsomal mixed function oxidase (MFO) activity. Drugs were administered intraperitoneally to male CDF_1 mice once daily for five days at a dose of 1 mg/kg. Activity of ethylmorphine N-demethylase, aniline hydroxylase, biphenyl hydroxylase, NADPH cytochrome c reductase and levels of cytochrome P-450 and microsomal protein were determined for 17 days following treatment. In addition, livers from treated and control mice were subjected to routine histopathological evaluation. The two drugs had divergent effects on MFO activity: Daunomycin produced increases in activity (15-30%) whereas Adriamycin produced decreases (15-30%). The inhibitory effect of Adriamycin had reached a nadir by 5-10 days post-treatment and had returned to normal by the seventeenth post-treatment day. The Daunomycin-stimulated activities were in some instances still increasing in magnitude by day 17 post-treatment. Although minor decreases in cytochrome P-450 were routinely seen with both drugs, a statistically significant decrease was recorded only for Adriamycin at one time point. Daunomycin produced consistent decreases in liver:body weight ratio, although neither liver protein nor glycogen levels were reduced. Histological examination of livers from treated mice showed no consistent drug-related alterations.

2. Preliminary experiments utilizing the three nitrosourea antineoplastic drugs, BCNU, CCNU and MeCCNU administered intraperitoneally in a four day treatment regimen, led to a striking increase in drug metabolizing enzyme activity from livers of only MeCCNU treated mice. In the doses employed, neither BCNU nor CCNU had any effect on the MFO system. In an attempt to further investigate the ability of MeCCNU to alter the activity of microsomal drug metabolizing enzymes in liver, mice

were treated with a single intraperitoneal dose of 40 mg/kg in an emulphor-based vehicle. All parameters studied showed a triphasic effect after MeCCNU treatment, with an increase in activity by 2 to 3 days post-treatment, a striking decrease in activity by 6 to 8 days post-treatment, and a subsequent increase again until 18 days after drug administration. At 2-3 days post-treatment, ethylmorphine N-demethylase and aniline hydroxylase were significantly increased (15-30%) while protein, NADPH cytochrome c reductase and cytochrome P-450 showed no significant change. Six to eight days after treatment, NADPH cytochrome c reductase, aniline hydroxylase, biphenyl hydroxylase and protein had decreased to 90% of control while cytochrome P-450 and ethylmorphine N-demethylase reached a nadir of 70% of control values eight days after drug administration. Ethylmorphine N-demethylase, biphenyl hydroxylase and NADPH cytochrome c reductase then showed an increase in activity to 30% (biphenyl hydroxylase and ethylmorphine N-demethylase) and 80% (NADPH cytochrome c reductase) above control by 17 days post-treatment while aniline hydroxylase, protein and liver to body weight ratio continued to be at or slightly below control values. Liver to body weight ratio was 15-20% below normal throughout the first eight days but had returned to normal by day 13. Cytochrome P-450 continued to be 30-40% below control levels at this time. All other parameters had returned to normal by this time.

B. Characterization of drug conjugating mechanisms in lungs of rabbits

Recent work in this laboratory has characterized and, to a large degree, defined the mixed function oxidase system of mammalian lung. Comparison of this system in lung and liver of rabbits revealed many similarities and few differences in terms of kinetics and general enzymology. In spite of the enormous importance of conjugation reactions in drug metabolism *in vivo* and in overall drug clearance, the conjugation of foreign compounds by lung tissue *in vitro* has received very limited attention. In an attempt to further characterize and compare the drug metabolizing potential of lung and liver, the subcellular localization and activities of several transferases were determined in rabbits using one or more substrates. Microsomal fractions were found to contain UDP-glucuronyltransferase activity while the supernatant fractions contained N-acetyltransferase, glutathione S-aryltransferase, and phenol sulfotransferase. With one exception, the enzyme activity in lung was considerably less than the activity toward the same substrate in liver and in several instances no activity was detected in lung. The activity of N-acetyltransferase toward the substrate *p*-aminobenzoic acid was equal in lung and liver, whereas the activity toward sulfadiazine was barely detectable in lung. UDP-glucuronyltransferase activity in lung, measured with *o*-aminophenol, was about 30% that of liver whereas the activity toward both *p*-nitrophenol and phenolphthalein was undetectable in lung. Similarly phenol sulfotransferase activity was not demonstrable in lung. Glutathione S-aryltransferase activity of lung was approximately 20% that of liver. Four day phenobarbital pretreatment produced increases in cytochrome P-450 and NADPH cytochrome c reductase in liver but no increase in any of the transferases in either organ. Pretreatment with 3-methylcholanthrene or SKF 525-A had no effect on any of the transferases studied.

C. Effects of activators of UDP-glucuronyltransferase activity in lung and liver

A wide variety of diverse materials have been demonstrated to activate hepatic UDP-glucuronyltransferase activity when added *in vitro* to enzyme incubations. In several instances, the combination of two or more activators produce additive or even synergistic effects. The precise molecular mechanisms responsible for such activation are not known but it has been established that in the presence of activators, latent enzyme activities can be unmasked and expressed, activities which in the "native" state would not be measurable.

As an extension of the project described in Section B above, we studied the effects of a number of activators on UDP-glucuronyltransferase activities in rabbit liver and lung. In particular, we were interested in whether or not the very low or undetectable activities in lung (see above) could be activated and whether the patterns of activation might be different in the two organs.

Microsomal fractions prepared from rabbit livers and lungs were employed as the enzyme sources. The following materials were used as potential activators, each in a wide range of concentrations so as to determine the optimum concentration for activation: mersalyl, digitonin, cetyl trimethyl ammonium bromide (CETAB), sodium deoxycholate (DOC), Triton X-100, sodium dodecyl sulfate (SDS), diethylnitrosamine (DEN), uridine diphosphate-N-acetylglucosamine (UDPNAG), and Mg^{++}. The effects of the activators were studied alone and in various combinations. Because previous reports had indicated differential effects based on the substrate employed for measuring UDP-glucuronyltransferase activity two substrates were employed, viz, *p*-nitrophenol, and *o*-aminophenol. The salient findings of this project may be summarized as follows:

1. All activators exhibited biphasic effects as a function of concentration, i.e., activation at low concentrations and inhibition at higher concentrations.
2. Mersalyl, digitonin and Triton X-100 were the most effective activators in liver. The effects of the detergents and UDPNAG were essentially additive. DEN reduced this activation. UDPNAG alone activated less than its combination with a detergent.
3. There were no significant differences in the effects of activators alone or in combination, with the two different substrates, *p*-nitrophenol or *o*-aminophenol.
4. None of the activators, alone or in combination unmasked measurable UDP-glucuronyltransferase activity in lung. This was true with both substrates.

D. The effect of storage, in vitro, on microsomal drug metabolism by mouse liver

Studies of microsomal mixed function oxidation have been hampered somewhat by the relative instability of the microsomal enzyme complex and the consequent need for enzyme preparation and assay on the same day. It also has been demonstrated that substantial differences exist in enzyme stability as a function of storage conditions, animal species, and the particular metabolic pathway in question. Prior to this study, there were no published reports dealing with the *in vitro* stability of the microsomal system in mouse liver preparations. Because of the general Program interest in the mouse, it was deemed useful to acquire information on microsomal enzyme stability in this species.

Mouse liver (CDF_1) microsomes were prepared, overlaid with isotonic buffered KCl and frozen at -30° for up to 20 days. Ethylmorphine N-demethylase and aniline hydroxylase were least stable, showing significant decreases in activity after 3 and 5 days of storage, respectively. NADPH cytochrome c reductase and cytochrome P-450 were both stable for at least 10 days. Electron micrographs of resuspended pellets after storage showed an increasing tendency toward aggregation of microsomal vesicles as the length of storage increased.

E. Biliary Excretion of Phenol Red

Studies of the biliary excretion of phenol red (PR) in normal Wistar rats and Gunn rats, deficient in UDP-glucuronyl transferase were completed during the current year. These studies have shown that the Gunn rat cannot form the glucuronide of PR which constitutes one-third of the total biliary PR output of normal rats. The pattern of biliary excretion of PR is consistent with first order elimination in both strains with $T_{1/2}$ = 38 min. in Wistar rats and $T_{1/2}$ = 91 min. in Gunn rats.

Development of a procedure for extraction from liver and serum of PR free of interfering materials permits the calculation of bile:tissue concentration ratios. Results indicate that in biliary excretion of PR by Wistar rats, free dye is concentrated sixty times and transfer from plasma to hepatocyte is apparently the rate limiting step. Gunn rats concentrate free biliary PR to only half the degree of Wistar rats.

F. Pharmacokinetic Model, Renal and Biliary Transport in Dogfish

For the past few years we have been exploring the possibility of using the dogfish shark as a model species for the rapid accrual of pharmacologic information concerning antineoplastic agents and other xenobiotics. The major rationale for this approach has been that both the rate of metabolism and rate of disposition of compounds administered to these animals might be lower than that of mammals.

In studies this year of about 20 drugs, the plasma half-times could be divided into three time catagories. The fastest (<20 min.) $t_{1/2}$'s occurred for nitrogen mustard, puromycin, and ellipticine. The next category of $t_{1/2}$'s are those in the intermediate range (20-60 min.). The drugs which occurred in this group are methotrexate, 6-mercaptopurine, actinomycin D, pseudourea, propanol-iminodi-dimethane sulfonate (PIDMS) and hycanthone. In the final category of agents with high $t_{1/2}$'s (61-130 min.) are found guanazole, cytoxan, imidazole-4-carboximide, cytosine arabinoside, dibromomannitol, 5-azacytidine, and diglycolaldehyde.

The following compounds were excreted at least 20% in 24 hours in the urine (values in parentheses are the actual amount excreted): puromycin (20), methotrexate (35), 6-mercaptopurine (21), PIDMS (44), imidazole-4-carboximide (24), and 5-azacytidine (20). An intermediary (7-17%) range of urinary excretion was observed for the following compounds: Actinomycin D (7), guanazole (15), cytoxan (12), cytosine arabinoside (17), dibromomannitol (12), and diglycolaldehyde (12). The lowest amounts of urinary excretion were seen for nitrogen mustard (4), ellipticine (1.4), 5-fluorouracil (3), pseudourea (4.4), and hycanthone (3.6). The biliary excretion for methotrexate (32%) was just slightly less than the amount appearing in the urine, while 9.6% of administered hycanthone appeared in the bile of the dogfish. Other drugs which appeared in detectable amounts in the bile were puromycin (5.6), cytoxan (3.5), dibromomannitol (2), and diglycolaldehyde (2). All of the other drugs were excreted in the bile in quantities of less than 1% of the administered dose.

These biliary values also were evaluated to see if there was a molecular weight biliary threshold in dogfish, similar to that proposed for mammals. In using the 24 hour biliary values such a threshold appears to exist starting at molecular weight about 260 (cytoxan) and increasing with pencillin (MW, 318), phenol red (MW, 354), methotrexate (MW, 454) and indocyanine green (MW, 775). It would appear that this dogfish model allows for the very rapid arrival (i.e. one summer) of such pharmacologic data since more than 20 drugs were studied using a minimum of 12 animals per drug.

Significance to Biomedical Research and the Program of the Institute:

As stated under objectives, drug interactions are known to profoundly influence the disposition of drugs and thus may determine whether a normal therapeutic dose of a drug will become either a subthreshold or toxic dose. The significance of this knowledge to cancer chemotherapy, particularly in the era of combination drug administration, cannot be overemphasized.

Proposed Course:

The projects being pursued are only partially complete. In addition to examining in more detail the influence of antineoplastic agents upon the metabolism, distribution, and binding of drugs and their toxicity, we plan to study the metabolic fate of antineoplastic agents. It is anticipated that we can make use of carcinolytic agents as model substrates for *in vitro* measurements of enzyme activity.

Publications:

1. Gram, T.E.: Separation of hepatic smooth and rough microsomes associated with drug-metabolizing enzymes. In Fleischer, S., Packer, L. and Estabrook, R.W. (eds.): *Methods of Enzymology*. Academic Press, NY. (in press) 1974.

2. Guarino, A.M., Reagan, R.L., and Gram, T.E.: Submicrosomal distribution of hepatic drug-metabolizing components in the Gunn rat. *Pharmacology* 10: 257-271, 1973.

3. Litterst, C.L., Reagan, R.L., and Gram, T.E.: Microsomal drug metabolizing enzyme activity in livers of bats. *Comp. Biochem. Physiol.* 47: 163-168, 1973.

4. Gram, T.E., Litterst, C.L. and Mimnaugh, E.G.: Comparative aspects of drug metabolism by mammalian lung and liver: mixed function oxidation and conjugation reactions. Proc. of an International Symposium on Drug Interactions (Mario Negri Instit, Milan, October 1973) Raven Press (in press), 1974.

5. Litterst, C.L., Mimnaugh, E.G., Reagan, R.L., and Gram, T.E.: Effect of storage on microsomal mixed function oxidase activity in mouse liver. Biochem. Pharmacol. (in press) 1974.

6. Gram, T.E., Litterst, C.L., and Mimnaugh, E.G.: Enzymatic conjugation of foreign chemical compounds by rabbit lung and liver. Drug Metab. Disp. (in press), 1974.

7. Litterst, C.L., Farber, T.M., and VanLoon, E.J.: Potentiation of CCl_4-induced hepatotoxicity in the dog by chronic exposure to phenobarbital. Tox. appl. Pharmacol. 25: 354-362, 1973.

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10. Homan, E.R. and Guarino, A.M.: Biliary excretion of phenol red by Wistar and Gunn Rats. Proc. Soc. Expt. Biol. Med. (in press) 1974.

11. Homan, E.R., Zendzian, R.P., Schott, L.D., Levy, H.B., and Adamson, R.H.: Studies on Poly I:C toxicity in experimental animals. Toxicol. Appl. Pharmacol. 23: 579-588, 1973.

12. Neil, G.L. and Homan, E.R.: The effect of dose interval on the survival of L1210 leukemic mice treated with DNA synthesis inhibitors. Canc. Res. 33: 895-901, 1973.

13. Guarino, A.M., Pritchard, J.B., Anderson, J.B., and Rall, D.P.: Tissue distribution on ^{14}C-DDT in the lobster after administration via intravascular or oral routes or after exposure from ambient wea water. Tox. Appl. Pharm. (in press) 1974.

Serial No. NCI-6123
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Program
July 1, 1973 through June 30, 1974

Project Title: Computer Methods for Quantification of Structure-Activity Relationships

Previous Serial Number: Same

Principal Investigators: A. M. Guarino and K. H. Ting

Other Investigators: H. Fales, T. E. Gram, R. C. T. Lee, K. C. Chu, G. W. A. Milne, M. B. Shapiro and J. M. Venditti

Cooperating Units: Drug Research and Development, Division of Cancer Treatment, Division of Computer Research and Technology, Laboratory of Chemistry and National Heart and Lung Institute

Man Years:
Total: 3.5
Professional: 3.5
Others: None

Project Description:

Ever since Meyer and Overton observed a correlation between lipid solubility and anesthetic potency of a series of drugs, many other physical parameters have been studied in an effort to discern other possible relationships between physical properties and a given pharmacologic effect. During the past year, one major project in this area has been continued. This project was designed to examine the relationships between the mass spectrum and pharmacologic activity of a drug molecule.

Principal Findings:

The possible relationship between mass spectra and pharmacologic activity was investigated with the help of various computer-based pattern-recognition techniques. By means of these techniques a datum point can be located on grid coordinates, and when many of these points are related in some way, they will cluster. In our experiments, designed to develop the necessary models, we studied the relationships between the mass spectra and the accepted pharmacologic activities of several drug classes. The present study is a small part of a binary classification tree we have constructed for this and future projects. The first classification starts with the question of whether or not a drug acts on the nervous system. If it does, then we ask if it is a stimulant; if not, then it is a depressant. This latter group is further classified as sedatives or tranquilizers and other groups are similarly categorized as narcotics, analgesics, hallucinogens, antineoplastic agents, etc. If one attempts to find whether some arbitrary unknown drug sample belongs to drug class A or B, a binary tree can be made from two binary classifiers in series. Classifier 1 is to distinguish "A and B" from "Not A and Not B". Only the unknowns that cluster with "A and B" are then sent to classifier 2. In order to build up an additional classifier 1, a training set consisting of 53 drugs of various pharmacological activities as a class (Not A, B) and 64 other drug samples were run through the learning machine. When a series of unknowns was studied, 85% of them were correctly classified. The mapping techniques, both non-linear mapping and projection, should enable humans, who are far better pattern recognizers than computers, to be involved in decision-making as to which drugs are worthy of expensive and time-consuming biologic testing. It is believed that this kind of man-machine interaction is likely to yield more productive results than a system in which the computer alone is required to make the final decision. It is noteworthy that pattern recognition techniques do not involve any prior knowledge of chemical structures or mass spectral rules. Therefore, although mass spectra were used as examples above, it is clear that the methods are general in scope and may be used as a tool for research on relationships which exist among any kinds of data which are not obviously correspondent. The fact that computer methods may predict pharmacologic activity of drugs from their mass spectra alone suggests that the foregoing is a new way of initial drug screening.

Significance to Biomedical Research and the Program of the Institute

Although the Drug Development Program makes extensive use of computers in the acquisition, storage and retrieval of information on the antineoplastic drugs, there has been little effort in the direction of using computers as learning and predictive devices. The present studies are based on the hypothesis that since the ultimate mechanism by which a drug exerts its pharmacologic or toxicologic action is by interaction of the drug molecules with macromolecular receptors in the body, then the mass spectral fragments pattern may indicate more precisely which parts of the drug molecule are necessary for the biologic action. Therefore, it is thought that once the mass spectral characteristics which are associated with good anticancer activity are established, the following can be done: 1) predict drug activity from the structure (mass spectrum); 2) estimate which compounds from a series of analogs would have the greatest oncolytic activity; 3) suggest to the chemist which structural features should be built into a drug molecule to obtain maximum antineoplastic activity, and 4) substitute "toxicity" data for "pharmacologic activity" and thus develop knowledge for understanding which structural moieties are toxic. Therefore one should be able to predict and synthesize drugs designed to minimize toxicity and enhance therapy.

Proposed Course

The mass spectra of groups of antineoplastic agents will be classified according to their relative potency in the L1210 screen. Correlations will be sought between the mass spectrum and biological activity with the aim of selecting those molecular features most prominently associated with oncolytic effects. Such studies may discover that other drugs may have properties suggesting they should be tested for possible greater clinical activity than those already in the clinic.

Publications

1. Ting, K.H., Lee, R.C.T., Milne, G.W.A., Shapiro, M.B. and Guarino, A.M.: Applications of Artificial Intelligence. Relationship Between Mass Spectra and Pharmacological Activity of Drugs. Science 180, 417-420, 1973.

2. Ting, K.H., Lee, R.C.T., Chang, C.L. and Guarino, A.M.: The Relationships between the Mass Spectra of Drugs and Their Biologic Activity - an Application of Artificial Intelligence to Chemistry, Computers in Biology and Medicine, in press, 1974.

SUMMARY REPORT

LABORATORY OF CHEMICAL PHARMACOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1973 - June 30, 1974

The efforts in the Laboratory of Chemical Pharmacology are concerned with elucidation of the pharmacological properties of potential and established antitumor agents. This includes the disposition, mechanism of action, structure-activity relationships, toxicity reversal, rational design and dose-response relationships of antitumor drugs and other model foreign compounds in laboratory animals and man. This requires in many instances the development of new methodologies to facilitate pharmacologic studies of antineoplastic agents whose disposition is not fully understood. Of special concern in disposition studies is the establishment of rates and rate constants which govern the passage of drugs across membranes from one body compartment to another, the assessment of drug biotransformation or binding to cellular constituents or macromolecules, and the determination of the rate of elimination of the drug and its metabolites. When this information is considered together with a drug's chemical and physical properties as well as its route and schedule of administration, predictions can be made concerning the effective concentration of a drug or its metabolites in tumor and susceptible normal tissue - the receptor sites. These studies are particularly important since one of the major factors contributing to the lack of adequate drug response of the host is the failure of the drug to reach the target site in sufficient concentration to eradicate the tumor cells. The knowledge gained from such studies when considered together with biochemical, toxicologic, and cytokinetic data will hopefully permit the design of drug dose-levels and schedules by the clinician for more effective therapeutic use of antineoplastic agents in human malignancies.

In addition, another major effort of the laboratory is to determine, develop and evaluate specific biochemical materials which are products of tumor cells which can be used for diagnostic purposes and for the assessment of the antitumor effects of various therapeutic modalities.

During the past year a number of research projects were continued and several new studies were initiated. Some of the experimental findings are high-lighted below.

The folic acid antagonists continue to be studied in this laboratory and studies this past year revealed that with constant infusion of MTX (2×10^{-8}M plasma MTX) deoxyuridine incorporation into DNA of small intestine was inhibited almost completely. Femur marrow and primary tumor although initially inhibited to some degree by this same concentration recover even in the presence of the drug. Concomitant studies using thymidine incorporation into DNA at this concentration of plasma MTX indicated no inhibition of incorporation of this deoxynucleoside. At plasma concentrations ten times higher (2×10^{-7}M) both deoxyuridine and thymidine incorporation into DNA of the small intestine were substantially inhibited. Recovery of all these tissues occurred at this plasma concentration of MTX if it was lowered rapidly at a time no longer than 10 hours. The action of MTX appeared to be dual and depended on plasma concentration in vivo. At plasma concentrations of 2×10^{-8}M, its inhibition of dihydrofolate reductase appeared to manifest itself through the inhibition of the tetrahydrofolate - requiring conversion of deoxyuridylate to thymidylate, since the incorporation of added labeled deoxyuridylate into DNA was inhibited while the incorporation of added labeled thymidylate was not. At higher concentrations however (around 2×10^{-7}M), inhibition of labeled thymidylate incorporation into DNA was also observed, a result which could be due to depletion of tetrahydrofolate cofactors required for other reactions necessary for DNA biosynthesis, such as purine and amino acid biosynthesis, or alternatively, to direct action by methotrexate at a second enzyme or site other than dihydrofolate reductase, but also essential for DNA biosynthesis.

In vitro transport studies of MTX using human AML and ALL cells and normal marrow demonstrated that there was no apparent difference between ALL cells and AML cells with respect to MTX influx velocity or net uptake. The steady state MTX levels were enhanced by vincristine at 10^{-5}M and 10^{-7}M and an average of 41% (range: 25-69) and steady state levels were reduced 18-30% by both hydrocortisone and cephalothin. Normal marrow studies with 10 volunteers indicated a 3-4 fold increase in steady-state intracellular levels of MTX in "immature" cell fractions as compared to mature granulocytes. Additionally, non-effluxable drug levels were also slightly higher in the "immature fraction".

Serum and cerebrospinal fluid antifolate levels were determined in patients receiving intrathecal methotrexate therapy and results indicated significantly elevated spinal fluid concentrations ($p < .05$) in patients 1) of large body surface area, 2) with overt meningeal leukemia, and 3) with adverse neurologic reactions during intrathecal methotrexate therapy. Also, low spinal fluid levels ($p < .05$) were noted in patients 1) of small body surface area, 2) receiving prophylactic intrathecal chemotherapy, and 3) with short remissions from central nervous system leukemia. Therefore during the past year, all patients receiving intrathecal MTX on the Pediatric Oncology Branch of the NCI were monitored for cerebrospinal fluid antifolate concentrations, and each patient's dosage was adjusted relative to that patient's levels. A significant reduction ($p < .05$) in the incidence of neurotoxicity has been observed during this period in comparison to the incidence noted prior to dosage modification. Several patients with very low spinal fluid levels had augmentation of their dosage to as much as 24 mg/m^2/dose without apparent neurotoxicity. Whether prolongation of remission duration can be achieved with dosage modification based on monitoring individual patient methotrexate concentrations remains to be established.

Determination of MTX pharmacokinetics and time course of inhibition of ^{3}H-UdR incorporation has been performed in patients with ovarian carcinoma. Five patients with ovarian carcinoma were studied thus far, each receiving a standard dose of 25 mg/m^2 IV. Thereafter serial in vitro studies of ^{3}H-UdR incorporation into DNA were performed on bone marrow and ascites cell samples and the results of these incorporation studies were correlated with the plasma and ascites levels of MTX. The major findings regarding pharmacokinetics were a three-phase disappearance from plasma with a terminal 1/2 life of 12 hours to 72 hours. The longer third phase correlated with the presence of partial renal obstruction and marked myelosuppression. Preferential accumulation of drug in ascites was observed in two patients. Efforts to define binding protein in ascites fluid by equilibrium dialysis were unsuccessful. In addition there appeared to be no binding protein capable of reducing the cytocidal effect of MTX in tissue culture.

The bone marrow of patients with ovarian cancer recovers from inhibition by MTX at plasma levels of 10^{-8}M, or approximately the same threshold as mouse bone marrow. DNA synthesis in ascites cells was only briefly and variably inhibited by MTX despite high levels in the ascites, indicating biochemical resistance of these cells to MTX. Tumor sensitivity has varied widely and further work is planned in patients with ovarian cancer to correlate DNA synthesis in bone marrow and in tumor.

Dialkyl esters of methotrexate with chain lengths from 1 to 8 carbons continue to be examined for their antitumor activity,effects on dihydrofolate reductase, and pharmacologic disposition. In vivo, in mouse and rat, the dialkyl esters were rapidly hydrolyzed to the parent compound, methotrexate, and their antitumor and other pharmacological properties therefore resembled those of the latter drug. Recent in vivo studies have therefore been carried out in larger mammalian species (dog and monkey), in which rapid hydrolysis does not occur. Physiological disposition studies in the dog with ^{3}H-diamyl-MTX (0.2 mg/kg) showed a biphasic plasma decline after intravenous administration, with half-times of 30 min and 105 min respectively. Excretion was mainly (> 60%) by the biliary route, in contrast to MTX, which is excreted largely by the kidneys. Tissue distribution was similar to that of methotrexate, with high levels being observed in liver and skin; penetration of the drug into cerebrospinal fluid was slow after systemic administration. Unlike methotrexate, diamyl MTX was rapidly metabolized; the principal metabolite (> 93%) was not MTX, but a compound which retains lipid solubility but differed in its chromatographic properties from the parent ester. Studies on the identity of this metabolite are in progress.

In the dog, intracisternally administered diamyl MTX was retained in the CSF for prolonged periods and gave rise to drug concentrations several-fold higher than those seen with equimolar doses of MTX. The disparity in tissue drug levels achieved appeared to be due to differing rates of transfer of the two drugs from CSF to plasma.

Disposition (absorption, distribution, metabolism and excretion) studies were undertaken with several new antitumor agents and potential antitumor agents. Among the agents investigated in this regard were 2,6-dimethyl-4-benzyl-4-demethylrifampicin (DMB), an acridine derivative NSC-141,549, inosine dialdehyde NSC-118,994, and DL-alanosine. In addition a systematic investigation of the role of the lymphatic system in the absorption and distribution of antitumor agents began.

DMB, an inhibitor of reverse transcriptase, either unlabeled or labeled with ^{14}C in the C-38 position, was administered to dogs at 1 or 5 mg/kg orally or intravenously. The compound was readily absorbed from the gastrointestinal tract, with plasma levels within the therapeutic range (> 5 μg/ml) being observed in the dog after oral administration of 1 mg/kg. In all three species, plasma disappearance of DMB administered by the oral, i.p. or i.v. routes was extremely slow (t 1/2: 2-4 days). In distribution studies in rats, 24 hr after an i.p. injection of ^{14}C-DMB (10 mg/kg), high tissue/plasma ratios were found in liver (6.0), pancreas (3.7), and adrenals (7.1); tissues with low levels included fat, muscle, and brain. The major excretory route was biliary, yet only 4.1 ± 0.4% of the dose (mean ± SE) was found in bile within 24 hr in the rat (10 mg DMB/kg; N = 4); bile radioactivity was found on thin-layer chromatography to consist largely of unchanged DMB and the corresponding DMB-naphthoquinone, although lesser amounts of three metabolites were detectable. No circulating metabolites, other than trace amounts of the naphthoquinone form of DMB, were detected by thin-layer chromatography of serum extracts. The slow plasma disappearance of circulating DMB in all species examined may be a consequence of plasma protein binding: the DMB: albumin association constants at pH 7.4 were 2.3×10^4, 3.5×10^5, and 7.6×10^4 M^{-1} for dog, rat, and bovine plasma albumin respectively. The major significant features of the physiological disposition of DMB in the mammalian species studied are its extremely slow plasma disappearance, its tight plasma protein binding, its prolonged tissue retention, and its excretion primarily by the biliary rather than the urinary route.

NSC-141,549 is a new antitumor agent having activity against leukemia L1210 and B-16 melanoma. The pharmacologic disposition of NSC-141,549 (MeAc) labeled in the 9-carbon position of the acridine ring was studied in mice and rats. The major route of excretion was biliary, with 45-70% of ^{14}C eliminated in the first 6 hours following i.v. administration. Transport into the bile appeared to be active, and the transport system saturable with therapeutic doses. The majority of the biliary ^{14}C was associated with one metabolite that could be separated by selective extractions and thin-layer chromatography followed by gel filtration. A minor portion of the dose was excreted in the urine and contains unchanged MeAc. Urinary excretion of ^{14}C following intraduodenal administration of bile from a ^{14}C-MeAc treated rat was compatible with the interpretation that reabsorption of the metabolite might occur via the entero-hepatic circulation. Biphasic plasma ^{14}C curves were obtained following i.v. administration and were characterized by a plateau preceded by a more rapid exponential decay. The level of ^{14}C in the plateau region was a linear function of the dose administered. Comparable plasma plateau ^{14}C levels could be obtained by either i.p. or oral administration to mice.

Inosine dialdehyde (IdA), the periodate-oxidation product of inosine was found to be toxic in vitro against leukemia L1210 and highly active against L1210 in mice with increases in life span of greater than 300%. It was also active against P-388 and several drug-resistant tumor lines in mice including those resistant to cytosine arabinoside, methotrexate, vincristine, hydroxyurea or 6-mercaptopurine.

Pharmacologic disposition of $[8]^{14}C$-IdA was studied in mice, rats, dogs and monkeys. In each of these species excretion occurred predominantly by the urinary route following either i.p. or i.v. injection. Urinary excretion is rapid with 35-59% eliminated during the first 3 hours and a total of 58-68% in 24 hours. The majority, if not all of the urinary ^{14}C was unchanged IdA. Biliary excretion was determined in rats and was found to be minor, accounting for 5% of the administered dose in 24 hours. In monkeys, dogs, and rats the plasma ^{14}C curves were biphasic having initial t 1/2 values of 12-24 minutes followed by a plateau and very gradual decline. The character of this curve was due to an increasing proportion of IdA binding to plasma proteins. Treatment of plasma samples with cold 5% TCA indicated 60-100% of plasma ^{14}C to be TCA insoluble and non-dialyzable; the proportion of ^{14}C bound in this manner increased with time after dose administration. Likewise, the ^{14}C of tissue homogenates prepared 24 hours after dose administration was TCA insoluble and non-dialyzable. ^{14}C could be released from these samples by digestion with trypsin or by treatment with HCl or acetic acid. In vitro chromatographic experiments indicated that IdA interacts with proteins and those amino acids possessing primary amino groups. Tissue culture studies demonstrated a decrease in IdA toxicity following interaction of IdA with protein.

Alanosine [2-amino-3-(N-hydroxy-nitrosamino)propionic acid] is a new antiviral and antitumor agent whose mechanism of action is not understood and for which no information is available as to its metabolic fate. Therefore, the metabolic disposition of 1-^{14}C-DL-alanosine was studied in several species. The kidneys were found to be the major excretory organ with a total of 42-75% ^{14}C eliminated in twenty-four hours following intraperitoneal, intravenous, oral, or subcutaneous administration to mice, rats, dogs, or monkeys. Also, in mice and rats, approximately 20% and 10%, respectively, of the ^{14}C dose was expired as $^{14}CO_2$. Studies in vitro suggested that the decarboxylation was an enzymatic process occurring predominantly in the liver. DL-alanosine was not excreted entirely unchanged in the urine and chromatographic analysis is presently being performed to identify several metabolites. In addition, the metabolites will be tested in several tumor systems to ascertain their potential activity. This data will be useful in designing clinical trials of DL-alanosine, or its metabolites, and for studying the clinical pharmacology of this compound.

The lymphatic absorption and distribution of various radioactive antitumor agents in the rat was investigated. Results thus far have demonstrated guanazole, dibromomannitol, diethylstilbestrol, estradiol and testosterone, are distributed in the body water and appear in lymph in quantities no greater than 5% of the administered dose. These results are similar to those obtained with antipyrine, a compound known to distribute in body water.

Other compounds, such as the antitumor agent o,p-DDD, attain concentrations in lymph 10-fold or greater than in plasma, representing 25% or more of the administered dose. In order to establish some of the characteristics required for the preferential lymphatic absorption of antitumor agents, a comparative study of structural analogs of o,p-DDD is underway. Although similar in molecular weight, the compounds in this series show marked differences in lipid solubility and in the extent to which they are metabolized, deposited in body fat, excreted in bile and urine, and carried to lymph. These studies will provide the background information to be used in attempts to enhance the absorption and distribution of antitumor agents by lymphatic channels. This data is of importance since metastases occur not only via the blood but also via the lymphatic system.

Several enzymes of interest to cancer chemotherapy have been studied this past year including thymidylate synthetase (for dUMP assay), cytidine deaminase, aldehyde oxidase and xanthine oxidase.

A new enzymatic assay for 5-FdUMP, the active metabolite of 5-FU, has been developed and has proved highly sensitive and specific. Conditions for the assay in terms of substrate (dUMP) concentration, temperature, and enzyme concentration have been defined and the assay has been used to define the intracellular conditions for suppression and recovery of DNA synthesis in mice bearing the P1534 leukemia. These studies demonstrated a characteristic recovery of UdR-incorporation into DNA at substrate/inhibition (dUMP/FdUMP) molar ratios of 10^4 for normal tissues and 10^5 for the tumor line. Recovery of DNA synthesis appear to be related mainly to a progressive increase in dUMP levels rather than a fall in FdUMP, the levels of which decline quite slowly during their final phase of intracellular disappearance. The relationship between substrate, inhibitor, and target enzyme levels in determining final enzyme activity has been confirmed by _in vitro_ studies which have duplicated the interrelationships of these variables to percent total enzyme activity observed _in vitro_. These analytical methods appear applicable to the general problem of enzyme inhibition by tight-binding inhibitors, and quantitatively show the importance of enzyme and substrate levels in determining drug effect.

Aldehyde oxidase and xanthine oxidase both catalyze the oxidation and pharmacological inactivation of a number of N-heterocyclic antitumor agents; inhibitors for these two enzymes _in vivo_ are of both theoretical and practical interest to the chemotherapy program. With reference to the nitroreductase activity of these two enzymes, nitro compounds are widely distributed in the environment, and are readily reduced _in vivo_ to hydroxyamino, nitroso, and amino derivatives. The reduced forms of certain nitro compounds are known to possess activity as carcinogens. Study of the nitroreductase activity of aldehyde oxidase and the assessment of its quantitative significance in nitro group reduction _in vivo_ can thus lead to information of potential relevance to the etiology of neoplastic disease. A series of substituted acridine derivatives has been found with K_i-values as inhibitors of mammalian aldehyde oxidase in the range 10^{-8} to 10^{-9}M. These agents represent the most potent aldehyde oxidase inhibitors described to date. Lesser inhibitory activity has been observed with a group of pyrazolopyrimidine derivatives, with K_i-values in the range of 10^{-6} to 10^{-7}M. In studies with nitro compounds as electron acceptors, nitrofurazone, 2-bromo-5-nitrothiazole, and the carcinogen 4-nitroquinoline-N-oxide were readily reduced by aldehyde oxidase under anaerobic conditions to the corresponding hydroxylamino compounds; a variety of other nitro compounds, including p-nitrobenzoate, did not show detectable acceptor activity. Effective electron donors for the reduction included aldehyde substrates such as butyraldehyde and benzaldehyde, and N-heterocyclic substrates such as 2-hydroxypyrimidine, 3'5'-dichloromethotrexate, and N-methylnicotinamide. NADH and NADPH failed to show detectable electron donor activity. The nitroreductase activity of aldehyde oxidase was significantly inhibited (> 50%) by menadione (15 μM) and by Triton X-100 (7 x 10^{-4}%); in contrast to that of xanthine oxidase, however, it was not inhibited by 4-hydroxypyrazolo (3,4-d)pyrimidine (allopurinol) (5 μM).

The enzyme cytidine deaminase inactivates cytosine arabinoside (ara-C). Recent studies have shown that increased levels of this enzyme may be induced by ara-C in patients with acute myelocytic leukemia, and that increased deaminase may be responsible for the development of ara-C resistance in these patients. Little is known about this enzyme in AML cells at present; thus detailed studies of its biochemical properties were undertaken in hopes of characterizing its substrate requirements and regulation in leukemic cells.

Both the deaminase and kinase have been purified, the former 700-fold and the latter more than 40-fold. Both enzymes have Km's of approximately 10^{-5} for deoxycytidine. The deaminase has greater activity for cytidine than for CdR or ara-C, while the kinase has greatest activity for CdR and ara-C and less for CR. The deaminase is more stable in purified form than the kinase, the latter losing activity rapidly over a week's time. Cytidine deaminase appears to increase rapidly in activity with maturation, while kinase stays relatively stable during maturation, if not declining slightly. The kinase/deaminase ratio has been proposed as an important determinant of responsivenes to ara-C. Our initial efforts have defined conditions for the accurate assay of kinase activity in the presence of deaminase, using 10^{-4}M THU to inhibit the deaminase activity. The two enzymes can also be separated cleanly by DEAE ion exchange chromatography. The importance of separating cells into mature and immature fractions prior to determining the K/D ratio, which may vary as much as 50-fold in the two fractions was determined. The K/D value in the immature cells is the only one of importance since immature cells are the only ones in active DNA synthesis and thus affected by the drug. In order to study this question in a prospective fashion, we have set up a co-operative study with the Baltimore Cancer Research Center and are determining the K/D value before, during and after therapy.

Mechanism of action studies were begun with several new antitumor agents and agents used for treatment of diseases encountered in patients with neoplasms.

Several experiments have suggested that the anti-Pneumocystis carinii or anti-protozoal action of pentamidine and propamidine may be due to inhibition of dihydrofolate reductase in a manner reminiscent of folate antagonists such as methotrexate and pyrimethamine. The enzyme isolated from the protozan Trypanosoma cruzi was found to be much more sensitive to the aromatic diamidines than mammalian tissue enzymes. Therefore this selective sensitivity of protozoan enzyme to aromatic diamidines may be the basis of action of these drugs.

Monodansylcadaverine was found to inhibit the establishment of some experimental tumors and various experiments suggested that this was due to its high inhibitory activity in fibrin-cross linking, thus denying the tumor a fibrin network.

Mouse and human interferon were found to have antitumor activity - mouse interferon against a transplantable murine reticulum cell sarcoma but not against leukemia L1210 or P388. Interferons did not exhibit cytotoxicity in vitro. Human interferon was found to have activity in owl monkey HVS-induced leukemia producing temporary remissions but its mechanism of actio remains to be elucidated.

Studies of hycanthone in pregnant mice and rabbits demonstrated that this compound was embryotoxic in both species over a broad period of gestation; hycanthone was also teratogenic in both species, but its teratogenic effect were manifested only at a discrete interval during gestation. In the mouse this critical period was found to be on gestational day 7. On the basis of the type of malformation produced after treatment on this day, it was proposed that hycanthone interferes with the closure of the anterior neuropore. Concomitant cytogenetic studies with hycanthone have shown that in vitro this compound exerts potent clastogenic activity and markedly depresses the mitotic index of PHA-stimulated lymphocytes. Examination of chromosomes of maternal blood and marrow and of fetal liver following treatment with hycanthone, however, showed minimal drug effects. Thus the teratogenic effects of hycanthone are probably not related to damage to fetal chromosomes.

Several DNA polymerases were isolated, including a reverse transcriptase, as judged by current criteria, from the Walker 256 carcinosarcoma. These polymerases were all purified and characterized. Antitumor compounds activ in vivo were tested as possible inhibitors using in vitro assays. The results showed individual differences in the inhibition of the specific polymerases, but complete correlation with in vivo results was not evident.

The availability of a large breeding and experimental monkey colony (600 animals representing 5 species) continues to provide this laboratory and other cooperating units with a unique resource for comparative pharmacologi toxicologic, and biochemical studies in subhuman primates. Thus far seve of the chemicals under study have induced tumors to date. These include 3 nitrosamines: [diethylnitrosamine (DENA), 1-nitrosopiperidine (PIP), and dipropylnitrosamine (DPNA)], methylnitrosourea (MNU), cycasin and its aglycone MAM acetate, aflatoxin B_1, and procarbazine. Methylnitrosourea has induced squamous cell carcinomas of the oral cavity and gastrointestinal tract, and procarbazine has induced acute myelogenous leukemias and hemangiosarcomas while the other carcinogens have all induced primary liver cell carcinomas.

The serum alpha-fetoprotein (AFP) levels in biopsy-proven disease were scattered over a wide range of 100,000-fold variation, which is analogous to the range seen in human hepatocellular carcinoma. Monkeys with N-nitrosodiethylamine induced neoplasms had significantly higher levels of AFP than those induced with 1-nitrosopiperidine, cycasin or aflatoxin B_1. Serial determinations of AFP levels demonstrated a tendency for the AFP level in each monkey with liver tumor to plateau after reaching a level characteristic for that tumor.

The half-life of native serum AFP was measured under three different conditions: 1. Hepatoma serum injected i.v. into adult monkeys, 2. the post-partum decline in neonatal monkeys and 3. the decline in a hepatoma-bearing monkey following surgical removal of the tumor. Despite many obvious and subtle differences among these situations the results tended to corroborate a T 1/2 for AFP of 2.8 - 3.6 days. In both the neonatal and post-surgical removal of tumor there may be continued synthesis of AFP, perhaps declining in the neonatal monkeys and increasing in the monkey which develops recurrent tumor 1 month post-surgery. Further work on catabolic rates of AFP where there is a varied total body load, and establishment of rates of cellular synthesis, will allow the estimation of total body tumor cell number and the changes that take place as a result of various modalities of tumor therapy.

Synthesis of AFP was demonstrated in fresh biopsy specimens for 2-6 days after tumor removal. The synthetic rate measured by incorporation of ^{14}C amino acids varied but appeared linear for 2-6 days, and in experiments in which incorporation was measured during the first day it appeared to be linear. From this it would appear that *in vitro* synthesis during the 24-48 hrs following tumor removal approximated *in vivo* synthesis, although many other experiments using differing culture conditions are necessary before this assumption can be made.

Methods have been developed to evaluate whether or not specific materials are elevated in the blood or urine of cancer patients. Initially, patients with advanced disease were entered in these studies. Results indicated that specific tRNA degradation products are increased in the urine of some patients with cancer. Further studies are in progress to determine the extent of these elevations and the types of tumors associated with increased excretion. Polyamines also are elevated in urines of certain cancer patients. Further studies regarding methodology are necessary before final conclusions can be reached. Other potential markers now under study include: carcinoembryonic antigen (CEA), AFP, serum fucose (and other protein bound serum sugars), β-aminoisobutyric acid, Regan's isoenzyme, and hydroxyproline.

In addition to the basic laboratory research effort, each senior investigator shares the responsibility for the administration and supervision of one or more contracts with outside research institutions. The contracts are primarily concerned with development of analytical-biochemical methodology; the study of pharmacological disposition of antitumor drugs and their metabolites in man, laboratory animals, and in *in vitro* predictive systems;

the development of pharmacokinetic mathematical models; and the development of biological markers for detection of cancer and assessment of cancer treatment. Approximately 12 new agents are studied each year, each of which reflect the interest of the Division of Cancer Treatment Program area for developing antitumor drugs for the clinic. It is expected that the number of agents to be studied through the contract mechanism will increase with the expanding program on the national level.

Serial No. NCI-3502
1. Lab. of Chemical Pharmacology
2. Pharmacokinetics and Pharmacodynamics, Drug Metabolism and Biochemical Pharmacology Sections
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Folic Acid Antagonists

Previous Serial Number: Same

Principal Investigators: Daniel S. Zaharko, David G. Johns, Bruce Chabner, Werner Bleyer, Richard Bender, Richard H. Adamson, and Vincent T. Oliverio

Other Investigators: Robert L. Dedrick and Vernon Wong

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS

Man Years:

Total:	4
Professional:	2
Other:	2

Project Description:

Objectives:

1. To Evaluate the Pharmacokinetics and Effects of Folate Antagonists

   Use of pharmacokinetics and computer simulations of drug kinetics in order to gain further insight into the limitations and future potential of folic acid antagonists. To utilize these methods to study drug kinetics and effects in animals bearing solid tumors.

2. To Study the Transport of Methotrexate and Metabolites across Mammalian Cell Membranes

   Studies are continuing to evaluate the effects of certain commonly administered "drugs" on methotrexate (MTX) in L1210 murine leukemia cells and human leukemia cells *in vitro* as a model for studying potential drug interactions in man.

3. To Study Lipid-soluble Derivatives of Methotrexate for Potential Antitumor Activity

   When administered intrathecally, methotrexate is of value in the therapy of meningeal leukemia, a frequently observed complication of acute leukemia of childhood. When administered by the more convenient oral or intravenous routes however, the drug does not readily pass the blood-brain barrier. It is likely that the poor transport of methotrexate from the systemic circulation into the cerebrospinal fluid is a consequence of its physicochemical properties, since its lipid solubility is extremely low and the carboxylic acid groups of the glutamate moiety of the molecule are ionized at physiological pH. Thus lipid soluble derivatives will be synthesized and evaluated for antitumor effect.

4. To Study the Clinical Pharmacology of Intrathecal Methotrexate

   During the last decade, an increasing number of children have apparently been cured of acute leukemia. The primary reason for this progress is the addition of central nervous system therapy to all patients whether or not they have evidence for meningeal leukemia. However, intrathecal chemotherapy is only partially effective and frequently associated with serious neurotoxicity (10-30% of all patients). These limitations are attributable, in part, to the lack of pharmacokinetic data and the empirical use of intrathecal chemotherapy. These considerations have prompted evaluation of cerebrospinal fluid and serum drug concentrations in all patients receiving intrathecal MTX. MTX was chosen for study because it is the most widely used intrathecal chemotherapeutic agent.

Methods Employed:

Chromatographically pure MTX-3',5'-T was employed throughout. Drugs were administered by the intravenous, intraperitoneal, or intraduodenal routes. Standard surgical procedures and specimen collection techniques were employed throughout.

The methodology employed for short-term *in vitro* uptake and efflux studies of MTX in normal marrow and human leukemic blast cells was adapted from Goldman *et al*. (*J. Biol. Chem*. 243: 5007, 1968). Marrow separations were carried out using the method of Evans *et al*. (*J. Clin. Exptl. Med*. - to be published).

Extensive use of an enzymatic assay technique (Bertino *et al*. *Methods Med. Res*. 10: 297-307, 1964) was necessary both to measure plasma and tissue methotrexate and to determine the quantity of new enzyme synthesized in tissues during recovery following drug treatment.

Extraction procedures for tissue DNA analysis were those described by Schneider, (Methods in Enzymology 3: 680-684, 1957).

Other standard analytical, biochemical and pharmacological techniques were described in detail in previous annual reports.

Major Findings:

1. Methotrexate Kinetics and Toxicity

Further investigations with the constant infusion of MTX in Lewis Lung tumor bearing mice have shown that at 2×10^{-8}M plasma MTX deoxyuridine incorporation into DNA of small intestine is inhibited almost completely. Femur marrow and primary tumor although initially inhibited to some degree by this same concentration recover even in the presence of the drug. Concomitant studies using thymidine incorporation into DNA at this concentration of plasma MTX indicate no inhibition of incorporation of this deoxynucleoside. At plasma concentrations ten times higher (2×10^{-7}M) both deoxyuridine and thymidine incorporation into DNA of the small intestine are substantially inhibited. Recovery of all these tissues does occur at this plasma concentration of MTX if it is lowered rapidly at a time no longer than 20 hours. The action of MTX appears to be dual and depends on plasma concentration *in vivo*. At plasma concentrations of around 2×10^{-8}M it appears to exert its action mainly through inhibition of dihydrofolate reductase whereas at higher concentrations (around 2×10^{-7}M) it appears to be acting in addition on purine synthesis. Dihydrofolate reductase inhibition recovers more rapidly than the inhibition of purine synthesis once the inhibitor is removed from the plasma.

In mice at a single i.p. dose of 0.15 mg/kg, vincristine delays the recovery of deoxyuridine incorporation into DNA in the tumor, the femur marrow and the small intestine from a concomitant treatment with MTX.

Amphotericin-B had no effect on the pattern of onset or recovery of nucleoside incorporation into DNA when used in combination with MTX.

2. Transport Studies

a) Leukemic Cells: Results indicate that there was no apparent difference between ALL cells and AML cells with respect to MTX influx velocity or net uptake. The steady state MTX levels were enhanced in average of 41% (range: 25-69) by vincristine at 10^{-5}M and 10^{-7}M. Steady state levels were reduced 18-30% by both hydrocortisone and cephalothin.

b) Normal Marrow: Studies on 10 volunteers indicated a 3-4 fold increase in steady-state intracellular levels of MTX in "immature" cell fractions as compared to mature granulocytes. Additionally, non-effluxable drug levels were slightly higher in the "immature fraction".

3. Lipid-soluble Derivatives of Methotrexate

Di-n-alkyl esters of methotrexate with chain-lengths from 1 to 8 carbons have been prepared, and the pharmacological activity of these compounds assessed *in vivo* and *in vitro*. The dialkyl esters are effective inhibitors of mammalian dihydrofolate reductase; their inhibitory activity, however, is less than that of the non-esterified parent compound, methotrexate. Within the ester series, enzyme inhibitory activity increases with ester chain-length up to a maximum with the 4- and 5-carbon esters; further increases in chain-length are accompanied by a decrease in inhibitory activity.

In vivo, in mouse and rat, the dialkyl esters are rapidly hydrolyzed to the parent compound, methotrexate, and their antitumor and other pharmacological properties therefore resemble those of the latter drug. Recent *in vivo* studies have therefore been carried out in larger mammalian species (dog and monkey), in which rapid hydrolysis does not occur. Physiological disposition studies in the dog with ^{3}H-diamyl-MTX (0.2 mg/kg) show a biphasic plasma decline after intravenous administration, with half-times of 30 min and 105 min respectively. Excretion is mainly ($> 60\%$) by the biliary route, in contrast to MTX, which is excreted largely by the kidneys. Tissue distribution is similar to that of methotrexate with high levels being observed in liver and skin; penetration of the drug into cerebrospinal fluid is slow after systemic administration. Unlike methotrexate, diamyl MTX is rapidly metabolized; the principal metabolite ($> 93\%$) is not MTX, but a compound which retains lipid solubility but differs in its chromatographic properties from the parent ester. Studies on the identity of this metabolite are in progress.

In the dog, intracisternally administered diamyl MTX is retained in the CSF for prolonged periods and gives rise to drug concentrations several-fold higher than those seen with equimolar doses of MTX. The disparity in tissue drug levels achieved appears to be due to differing rates of transfer of the two drugs from CSF to plasma.

4. Clinical Pharmacology of Intrathecal Methotrexate

Cerebrospinal fluid and serum samples were obtained from 41 patients receiving intrathecal methotrexate therapy. After intrathecal injection of 12 - 15 mg/m^2, the half-disappearance time of methotrexate in the spinal fluid ranged from 2 to 5 hours during the day after administration and from 12-18 hrs thereafter, resulting in mean ($\pm$ 1 S.D.) antifolate levels of $1.3\ (\pm 0.9) \times 10^{-5}$ and $3.4\ (\pm 0.4) \times 10^{-8}$ molar at 12 and 72 hours, respectively. Serum methotrexate concentration peaked between 3 and 6 hours post-injection, declined slowly thereafter at an average half-life of 18 hours, and remained above 10^{-8} molar for more than 40 hours. Significantly elevated spinal fluid concentrations ($p < .05$) were observed in patients 1) of large body surface area, 2) with overt meningeal leukemia, and 3) with adverse neurologic reactions during intrathecal methotrexate therapy. Low spinal fluid levels ($p < .05$) were noted in patients 1) of small body surface area, 2) receiving prophylactic intrathecal chemotherapy, and 3) with short remissions from central nervous system leukemia.

During the past year, all patients receiving intrathecal MTX on the Pediatric Oncology Branch of the NCI were monitored for cerebrospinal fluid antifolate concentrations, and each patient's dosage was adjusted relative to that patient's levels. A significant reduction ($p < .05$) in the incidence of neurotoxicity has been observed during this period in comparison to the incidence noted prior to dosage modification. Several patients with very low spinal fluid levels had augmentation of their dosage to as much as 24 mg/m^2/dose without apparent neurotoxicity. Whether prolongation of remission duration can be achieved with dosage modification per individual patient methotrexate concentrations remains to be established.

Significance to Bio-medical Research and the Program of the Institute:

Among the active antitumor folic acid antagonists, MTX is the most widely used, and is clinically effective in the treatment of acute lymphocytic leukemia, choriocarcinoma, Burkitt's lymphoma, primary hepatocellular carcinoma, and epidermoid cancer of the head and neck. Because of the great clinical importance of MTX, a complete knowledge of its pharmacology and toxicology is of practical value. With this established agent it is envisioned that additional information on its distribution and metabolism in a variety of species, information on toxicity reversal by CF, and a further understanding of its uptake characteristics into malignant cells alone or in the presence of other drugs, will lead to its improved use and the design and use of newer more effective drugs.

Proposed Course:

1. Further investigations of drug kinetics and effect on solid tumor-bearing mice will be continued. Studies concerned with drug combinations given in sequence based on observed effects on DNA synthesis and knowledge of drug kinetics will be carried out.

2. Continued studies to characterize "normal" myeloid MTX uptake in marrow are in progress. Additionally, studies of the inhibitions of DNA synthesis as related to intra-cellular MTX levels are in progress.

3. Studies on the pharmacological activity of lipid-soluble dialkyl esters of methotrexate, and of other lipid-soluble folate antagonists, will be continued in several mammalian species, and toxicity studies will be initiated if warranted.

4. In all forms of metastatic malignancy, prolonged survival has been tempered by an increasing incidence of central nervous system involvement. Concomitantly, the use of IT MTX is rapidly increasing, not only for treatment (e.g. primary brain tumors, choriocarcinoma, lymphoma), but also for prophylaxis (e.g. Ewing's sarcoma). It is imperative that the pharmacokinetics of IT MTX, and other IT agents be more carefully delineated. The mechanism of neurotoxicity must be established, as well as the role of other factors affecting the pharmacology of the IT chemotherapy.

Publications:

1. Johns, D. G., Farquhar, D., Wolpert, M. K., Chabner, B. A., and Loo, T. L.: Dialkyl esters of methotrexate and 3',5'-dichloromethotrexate: Synthesis and interaction with aldehyde oxidase and dihydrofolate reductase. Drug Metab. Disposition 1: 580-589, 1973.

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8. Straw, J. A., Hart, M. M., Klubes, P., Zaharko, D. S., and Dedrick, R. L.: Distribution of anticancer agents in spontaneous animal tumors. J. Nat. Cancer Inst. 1974, in press.

9. Hansen, H. H. and Bender, R. A.: The cytocentrifuge and cerebrospinal fluid cytology. Acta Cytol. 1974, in press.

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11. Bleyer, W. A., Drake, J. C., and Chabner, B. A.: Neurotoxicity and elevated cerebrospinal-fluid methotrexate concentration in meningeal leukemia. New Engl. J. Med. 289: 770-773, 1973.

Serial No. NCI-3505
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Potential Embryotoxic, Carcinogenic and Other Adverse Effects of Anticancer Agents

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson, Susan S. Fabro and Vincent T. Oliverio

Other Investigators: Jacqueline Whang-Peng

Cooperating Units: Human Tumor Cell Biology Branch

Man Years:

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives:

The susceptibility of newborn and adult animals to environmental chemical carcinogens and particularly to drugs, including the antiviral agents, of interest in cancer chemotherapy is of vital necessity and of particular relevance to the program of the NCI. The characterizations and treatment of such induced tumors is of both theoretical and practical importance. The specific objectives are:

To determine the carcinogenic activity of chemicals to which the human population may be exposed, particularly drugs of interest in cancer chemotherapy, such as alkylating agents, antimetabolites, agents that intercalate with DNA, polymers and various antiviral agents and antibiotics.

To investigate the potential embryotoxic effects of anticancer agents and antiviral agents that may be used in patients of child bearing age with or without tumors.

To ascertain unusual pharmacologic and toxic properties of various antibiotics and antiviral agents which are used in patients with neoplastic disease.

To compare the susceptibility of newborn vs. adult animals to chemical carcinogens.

To characterize and transplant carcinogen-induced tumors as new tools for experimental cancer chemotherapy.

Methods Employed:

Usual biological, pharmacologic, biochemical and histopathologic techniques.

Major Findings:

Embryotoxicity of Antitumor Agents

Studies of hycanthone in pregnant mice and rabbits demonstrated that this compound was embryotoxic in both species over a broad period of gestation; hycanthone was also teratogenic in both species, but its teratogenic effects were manifested only at a discrete interval during gestation. In the mouse, this critical period was found to be on gestational day 7. On the basis of the type of malformation produced after treatment on this day, it was proposed that hycanthone interferes with the closure of the anterior neuropore. Concomitant cytogenetic studies with hycanthone have shown that in vitro this compound exerts potent clastogenic activity and markedly depresses the mitotic index of PHA-stimulated lymphocytes. Examination of chromosomes of maternal blood and marrow and of fetal liver following treatment with hycanthone, however, showed minimal drug effects. Thus the teratogenic effects of hycanthone are probably not related to damage to fetal chromosomes.

Similar studies with the antitumor antibiotic adriamycin are in progress. In these studies, adriamycin was given to pregnant mice at various periods of gestation, and at term samples of maternal marrow and blood as well as fetal liver was subjected to cytogenetic analysis. Additionally, samples of both maternal and fetal heart are examined electron-microscopically for adriamycin-induced lesions. The fetuses were examined for morphologic abnormalities with special emphasis on cardiac malformations. These studies are currently being carried out in mice and in the future will be extended to rabbits.

Evaluation of Agents for Carcinogenic Activity

Several interferon inducers including poly I·poly C and tilorone have been evaluated for potential carcinogenic effects. Data thus far suggest that these compounds are not highly carcinogenic in CDF_1 mice.

Hycanthone is presently under evaluation as a potential carcinogen in CDF_1 mice. Hycanthone is under evaluation because it is widely used in South America and Africa for the treatment of schistosomiasis and over 100,000 people have been exposed to the compound, and because it produces embryotoxic effects in the mouse and rabbit and chromosomal abberrations in human PHA-stimulated lymphocytes at high doses. The mice autopsied thus far, 1 year after dosing ceased, did not have tumors and thus there is little carcinogenic potential of hycanthone.

Procarbazine is under further evaluation as a carcinogen in several species both alone and in combination with other antitumor agents and X-radiation. This is similar to the combination chemotherapy protocol for patients with Hodgkin's disease. Procarbazine has been found to induce tumors in mice, rats and monkeys in our colony and may be suspected of being carcinogenic in humans when administered chronically.

Significance to Bio-medical Research and the Program of the Institute:

The possible carcinogenic activity of antitumor agents is not only of clinical importance, but theoretically may improve our understanding of the malignant process from a theoretical standpoint.

Several drugs used as anticancer agents are known to be carcinogens. Few, however, have exhibited such strong carcinogenic activity as procarbazine, and none elicit such a wide spectrum of tumors in mice and rats. With the clinical use of procarbazine, it is essential to carefully delineate its carcinogenic potential alone and in combination with other anticancer agents in both rodents and primates.

Evaluation of the possible embryotoxic and/or teratogenic effects of various anticancer agents will allow the clinician a more rational choice in the use of these agents in pregnant women with tumors and may also help define the nutritional requirements of the fetus.

Unusual pharmacologic and toxic effects of antiviral agents and antibiotics are of major importance to the clinician as these drugs are widely used in patients with neoplastic disease.

The wide exposure of the human population to hycanthone makes it necessary to evaluate the potential carcinogenic activity of this agent and also to evaluate if there is a dose response effect since it is generally administered in humans once at a dose of 3 mg/kg i.m.

Proposed Course:

New antiviral and anticancer agents of clinical interest will be evaluated as potential carcinogens. Tumors that occur from various carcinogens will be evaluated for their applicability to programs for antitumor testing. The pharmacologic and embryotoxic properties of antibiotics and antiviral agents used in patients with cancer will continue to be examined.

Publications:

1. Sieber, S. M., Whang-Peng, J., Johns, D. G., and Adamson, R. H.: Effects of hycanthone on rapidly proliferating cells. Biochem. Pharmacol. 22: 1253-1262, 1973.

This report was previously entitled, "Potential Carcinogenic Activity and Chronic Toxicity of Anticancer Agents".

Serial No. NCI-3506
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Pharmacology and Disposition of Alkylating Agents and Other Antitumor Drugs

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson, Susan Sieber Fabro and Richard Cysyk

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	3.0
Professional:	2.0
Other:	1.0

Project Description:

Objectives:

To study the metabolic fate of experimental antitumor agents prior to clinical trial and to ascertain whether their metabolites are active in various tumor systems.

To study the pharmacology of antitumor drugs of current interest in clinical chemotherapy in order to design better therapeutic regimens.

To evaluate the usefulness of new tumor systems in experimental chemotherapy.

To design newer anticancer drugs by rational approaches.

Methods Employed:

Usual pharmacologic, biochemical, analytical, immunologic and histopathologic techniques.

Major Findings:

Pharmacologic Disposition of NSC-141,549, an Acridine Derivative

NSC-141,549 is a new antitumor agent having activity against leukemia L1210 and B-16 melanoma. The pharmacologic disposition of NSC-141,549 (MeAc) labeled in the 9 carbon position of the acridine ring was studied in mice and rats. The major route of excretion is biliary with 45-70% of ^{14}C eliminated in the first 6 hours following IV administration. Transport into the bile appears to be active and the transport system saturable with therapeutic doses. The majority of the biliary ^{14}C is associated with one metabolite that can be separated by selective extractions, thin layer chromatography followed by gel filtration. A minor portion of the dose was excreted in the urine and contains unchanged MeAc. Urinary excretion of ^{14}C following intraduodenal administration of bile from a ^{14}C-MeAc treated rat suggested that reabsorption of the metabolite might occur via the entero-hepatic circulation. Biphasic plasma ^{14}C curves were obtained following IV administration and were characterized by a plateau preceeded by a more rapid exponential decay. The level of ^{14}C in the plateau region was a linear function of the dose administered. Comparable plasma plateau ^{14}C levels could be obtained by either IP or oral administration to mice.

Antitumor Properties and Pharmacologic Disposition of Ionosine Dialdehyde (NSC-118,994)

Inosine dialdehyde (IdA), the periodate-oxidation product of inosine was found to be toxic *in vitro* against leukemia L1210 and highly active against L1210 in mice with increases in life span of greater than 300%. It was also active against P-388 and several drug-resistant tumor lines in mice including those resistant to cytosine arabinoside, methotrexate, vincristine, hydroxyurea or 6-mercaptopurine.

Pharmacologic disposition of $[8]^{14}C$-IdA was studied in mice, rats, dogs and monkeys. In each of these species excretion takes place predominantly by the urinary route following either IP or IV injection. Urinary excretion is rapid with 35-59% eliminated during the first 3 hours and a total of 58-68% in 24 hours. The majority, if not all of the urinary excreted ^{14}C was unchanged IdA. Biliary excretion was determined in rats and was found to be minor, accounting for 5% of the administered dose in 24 hours. In monkeys, dogs, and rats the plasma ^{14}C curves were biphasic having initial t 1/2 values of 12-24 minutes followed by a plateau and very gradual decline The character of this curve was due to an increasing proportion of IdA binding to plasma proteins. Treatment of plasma samples with cold 5% TCA indicated 60-100% of plasma ^{14}C to be TCA insoluble and non-dialyzable; the proportion of ^{14}C bound in this manner increased with time after dose administration. Likewise, the ^{14}C of tissue homogenates prepared 24 hours after dose administration was TCA insoluble and non-dialyzable. ^{14}C could be released from these samples by digestion with trypsin or by treatment with HCl or acetic acid. *In vitro* chromatographic experiments indicated that IdA interacts with proteins and those amino acids possessing primary amino groups. Tissue culture studies demonstrated a decrease in IdA toxicity following interaction of IdA with protein.

Disposition of Guanazole

Guanazole was effective against various animal tumor systems and markedly more active when given frequently than on a once per day schedule. Studies in our laboratory suggest that one reason for the necessity of frequent dosing with guanazole is that it was rapidly excreted by the mouse and rat with 80-90% appearing in the urine within 3 hours. Tissue distribution studies also revealed that ^{14}C-guanazole was found in organs involved in urinary excretion and did not readily cross the blood-brain barrier of mice or dogs. This was not surprising in light of the fact that guanazole is highly polar having a heptane or chloroform/aqueous partition coefficient of <0.0001. Nevertheless, guanazole was adsorbed after oral administration to the mouse, albeit incompletely, since 70% of the radioactivity appeared in the urine 4 hours after administration with only 6% in the feces. Chromatographic studies of the urine from mice, rats, and dogs treated with ^{14}C-guanazole did not reveal any radioactive compounds other than guanazole. In addition, no $^{14}CO_2$ was found in the expired air after administration of ^{14}C-guanazole to mice. This information along with the results that guanazole is cytotoxic _in vitro_ to four cell lines, that antitumor doses used _in vivo_ could be extrapolated from the cytotoxic concentrations used _in vitro_, and the structural specificity of the 3,5-diamino substitution for cytotoxic activity, strongly suggest that the active antitumor molecule is guanazole _per se_ and this is excreted unchanged by various animal species. In addition, studies of ^{14}C-guanazole in man revealed similar findings to the above in that 60% of the radioactivity was recovered at the end of 24 hours infusion and 91% recovered after 80 hours. Again chromatographic studies revealed that the majority, if not all the radioactivity found, was unchanged guanazole.

Disposition of ^{14}C DL-Alanosine

Alanosine [2-amino-3-(N-hydroxy-nitrosamino)propionic acid] is a new antiviral and antitumor agent whose mechanism of action is not understood and for which no information is available as to its metabolic fate. Therefore, the metabolic disposition of 1-^{14}C-DL-alanosine was studied in several species. Results identified the kidneys as the major excretory organ with a total of 42-75% ^{14}C eliminated in twenty-four hours following intraperitoneal, intravenous, oral, or subcutaneous administration to mice, rats, dogs, or monkeys. Also, in mice and rats, approximately 20% and 10%, respectively, of the ^{14}C dose was expired as $^{14}CO_2$. Studies _in vitro_ suggested that the decarboxylation was an enzymatic process occurring predominantly in the liver. DL-alanosine was not excreted total unchanged in the urine and chromatographic analysis is presently being performed to identify several metabolites. In addition, the metabolites will be tested in several tumor systems to ascertain their potential activity. This data will be useful in designing clinical trials of DL-alanosine, or its metabolites, and for studying the clinical pharmacology of this compound.

Studies of Lymphatic Absorption and Distribution of Antitumor Agents

The lymphatic absorption and distribution of various radioactive antitumor agents in the rat was investigated. Results thus far have demonstrated guanazole, dibromomannitol, diethyl-stilbestrol, estradiol and testosterone, are distributed in the body water and appear in lymph in quantities no greater than 5% of the administered dose. These results are similar to those obtained with antipyrine, a compound known to distribute in body water. Other compounds, such as the antitumor agent o,p-DDD, attain concentrations in lymph 10-fold or greater than in plasma, representing 25% or more of the administered dose. In order to establish some of the characteristics required for the preferential lymphatic absorption of antitumor agents, a comparative study of structural analogs of o,p-DDD is underway. Although similar in molecular weight, the compounds in this series show marked differences in lipid solubility and in the extent to which they are metabolized, deposited in body fat, excreted in bile and urine, and carried to lymph. These studies will provide the background information to be used in attempts to enhance the absorption and distribution of antitumor agents by lymphatic channels. This data is of importance since metastases generally occurs not only by the blood but also via the lymphatic system.

Rational Approaches to Design of Anticancer Agents

Eleven biological and structural characteristics that might be useful in the design of new antitumor agents were evaluated. These characteristics were planar aromatic compounds with keto or quinone groups, compounds that induce interferon, antispermatogenic chemicals, compounds localizing in specific tissues or organs, dual chemotherapeutic agents, N-O-O triangulation, agents initiating the cell cycle, reverse transcriptase inhibitors, drug latentiation, analogs of non-essential amino acids, and chemical enhancing the host-immune response. Specific potential anticancer agents using these characteristics were designed and suggested for synthesis to the Drug Research and Development Branch. In addition, the rational for examining certain marine bacteria for antibiotics which may have antineoplastic activity was suggested.

Significance to Bio-medical Research and the Program of the Institute:

Knowledge of the metabolic fate of various anticancer agents, their plasma t 1/2, their route of disposition, and their interaction with other drugs will lead to better therapeutic treatment in man and will aid in the design of agents for experimental animal trials. A rational approach to the design, selection and testing of new anticancer agents is of utmost importance and supplements the screening approval of Drug Research and Development Branch. Careful evaluation of the effect of potential and known antitumor compounds in various tumor systems should be made until sufficient data is available to judge their usefulness in screening compounds for ultimate clinical trials.

Proposed Course:

1. Studies of the metabolic fate of new anticancer agents in animals and man will continue. In particular, studies will be intensified on the disposition of alanosine, inosine dialdehyde, and new agents identified as active in the screen.
2. The role of the lymphatic system in drug disposition in general and cancer chemotherapeutic agents in particular will be further investigated.
3. Studies on the mechanism of the antitumor action of interferon will continue and the antitumor activity of various interferon inducers and interferon *per se* will be evaluated in various models.
4. A rational approach to the design, selection, and testing of potential anticancer agents will continue.
5. Use of induced tumors (primary and transplanted) for chemotherapy will be continued.

Publications:

1. Adamson, R. H. and Hahn, M. A.: The disposition of the antitumor agent 3,5-Diamino-1,2,4-triazole (Guanazole) in mice, rats, and dogs. *Xenobiotica* 3: 247-256, 1973.
2. Adamson, R. H.: Oncology and the marine environment. In Worthen, L. R. (ed.): *Food-Drugs from the Sea Proceedings 1972*. Washington, D.C., Marine Technology Society, 1973, pp. 385-389.
3. Adamson, R. H., and Davies, D. S.: Comparative aspects of absorption, distribution, metabolism, and excretion of drugs. In Michelson, M. J. (ed.): *International Encyclopedia of Pharmacology and Toxicology Section 85*. Oxford and New York, Pergamon Press, 1973, Chapter 9, pp. 851-911.
4. Adamson, R. H.: Rational development of antitumor agents. In Okita, G. T. and Acheson, G. H. (eds.): *Proceedings of the Fifth International Congress on Pharmacology*. Basel, New York, S. Karger, 1973, Volume 3, pp. 402-410.

This report was previously entitled, "Pharmacology of Alkylating Agents and Other Antitumor Drugs".

Serial No. NCI-3509
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Carcinogenesis, Chemotherapy and Biological Markers in Primates

Previous Serial Number: Same

Principal Investigator: Richard H. Adamson

Other Investigators: Robert McIntire, Susan S. Fabro, T. Phillip Waalkes, Charles L. McIntosh, Pelayo Correa and Dan Dalgard

Cooperating Units: Laboratory of Cell Biology; NCI; Clinic of Surgery, NHLI; North Florida Regional Hospital, Gainesville, Fla.; and Hazleton Laboratories, Inc., Falls Church, Va.

Man Years:

Total:	3.5
Professional:	2.5
Others:	1.0

Project Description:

Objectives:

This project is designed to obtain comparative data on the response of primates and rodents to known carcinogens and to materials suspected to be carcinogenic in man and to develop model primate tumor systems predictable for therapy for man and for development of biological markers. Specifically the objectives are:

To obtain data on which to base a comparison of the response of monkeys and rodents to materials known or suspected to be carcinogenic to man, and thereby to evaluate the relative merits of rodents and primates in predicting for man.

To study the effects of compounds, generally of potential carcinogenic activity in newborn primates and to determine if the newborn primate, like the rodent, will be highly susceptible to carcinogenesis.

To develop model non-human primate tumor systems.

To evaluate the long-term effects of anticancer drugs which are being used clinically for long-term remissions and for diffuse collagen disorders.

To develop biological markers which will aid in the diagnosis of preneoplastic changes as well as for existing neoplasia.

To use normal and tumor-bearing primates for studying the pharmacological, toxicological, and chemotherapeutic properties of various anticancer, antiviral, and antimicrobial agents.

To use the various tumors and animals with premalignant changes and with tumors for biochemical and immunological studies.

To evaluate known and suspected oncogenic virus in subhuman primates and to utilize the tumors induced for chemotherapy with antiviral and antitumor agents.

Methods Employed:

Usual pharmacologic, biochemical, clinical, and anatomical pathologic techniques and cytological and immunologic techniques as well as breeding and nursing care of infant monkeys and special dietary regimens. The breeding record and health status of the breeding colony is similar to that reported for previous years.

Major Findings:

General Information:

The present colony consisting of 600 animals is comprised of five species: Macaca mulatta, Macaca fascicularis, Cercopithecus aethiops, Galago crassicaudatus, and Aotus trivirgatus. Of these 450 are experimental; the remaining 150 are adult breeders which supply the newborns for experimental study.

Previous annual reports have contained detailed information concerning the management and maintenance procedures employed in the breeding colony and information concerning the methods utilized in treating the newborns. These procedures have not been significantly altered and are, therefore, not included in this report.

Various physical hematological and chemical parameters are monitored in each animal to evaluate the general health status and any evidence of tumorigenesis. Surgical biopsies of tumor specimens are obtained when indicated and submitted for histopathologic evaluation. All animals which die or are sacrificed are carefully necropsied and tissues examined microscopically.

No major disease problems have been encountered during this period.

Newborn monkeys are generally used with the hope that the infant primate like the infant rodent will be more susceptible than the adult to chemical carcinogens. Newborn M. mulatta, M. fascicularis, and C. aethiops are separated from their mother a few hours post-partum, placed in a 24 hour nursery and put on a carcinogenic regimen. In some cases, initiation of compound may be delayed until naturally occurring neonatal levels of AFP have declined to normal background levels. The compounds are administered subcutaneously, intravenously, intraperitoneally, or orally at varying dose levels and frequencies. Administration of the carcinogen is continued for extended periods generally ranging from 5 to 7 years and the animals held for an additional observation period of 6 years.

The principle assay used for AFP determinations is the Ouchterlony double diffusion technique which is able to detect levels in excess of approximately 400 ng/ml. Radioimmunoassay is used for specific studies where more quantitative data are needed. Although simian and human AFP do cross-react antisera rendered mono-specific for simian AFP was used. Normal AFP ranges for man and monkeys are similar, being approximately 12 ng/ml.

Compounds Inducing Tumors:

Seven of the chemicals employed have induced tumors to date in the three species of monkeys under study - these include 3 nitrosamines: [diethylnitrosamine (DENA), 1-nitrosopiperidine (PIP), and dipropylnitrosamine (DPNA)], methylnitrosourea (MNU), cycasin and its aglycone MAM acetate, aflatoxin B_1, and procarbazine. Methylnitrosurea has induced squamous cell carcinomas of the oral cavity and gastrointestinal tract, and procarbazine has induced acute myelogenous leukemias and hemangiosarcomas while the other carcinogens have all induced primary liver cell carcinomas. A summary of the number of primary hepatic cell carcinomas induced thus far is presented below.

CHEMICALS INDUCING PRIMARY LIVER TUMORS IN NON-HUMAN PRIMATES

Chemical and Route of Administration	No. with Tumor	No. Treated	% Incidence
DENA - oral	30	37	81
DENA - IP	77	77	100
DPNA - IP	6	6	100
PIP - oral	9	11	82
MAM acetate - oral and IP	4	22	18
Aflatoxin B_1 - oral and IP	3	35	9

The carcinogen studied most extensively is DENA. When given intraperitoneally in gradually increasing doses of 10, 20, and 40 mg/kg every 14 days a tumor incidence of 100% can be anticipated. The average induction time is 15 months with a range of 6 to 22 months.

The AFP response in these animals as determined by double diffusion is quite variable in that some animals become positive after 5 - 6 months of compound administration, whereas others may take 16 - 18 months. Once they are positive however, the AFP level escalates rapidly. Exploratory laparotomies done on 30 animals shortly after the first positive AFP determination revealed no gross evidence of tumor. Histologically, toxic hepatitis and hyperplastic nodules were commonly seen at this stage, but tumors were not detected. Six animals were euthanized and approximately 100 liver sections from each animal were examined for evidence of tumor. No tumors were detected. In those animals in which compound administration was continued, the AFP level continued to increase and tumor was generally present 5 - 7 months later. The AFP level at this point apparently is a combined function of the hepatotoxic action of DENA and the rate of synthesis by the developing tumor nodules. When DENA is discontinued in a tumor-bearing animal, the AFP level may drop temporarily and then continue to increase at a rate dependent upon the tumor load and the composite rate of synthesis by the individual nodules. Administration of DENA is generally discontinued when tumor is confirmed by histopathologic examination.

The gross and histologic picture of early tumor varies from 1 - 2 isolated nodules surrounded by relatively normal liver to numerous small nodules, cirrhosis, toxic hepatitis and necrosis. Tumor generally continues to invade the remaining liver until the entire organ is replaced by tumor. Metastasis to the lungs is common in animals with advanced tumor, whereas metastatic tumor in other locations occurs infrequently. Death or euthanasia because of unmanageable tumor generally occurs by 24 months of age. The AFP commonly reaches levels of 5000 mcg/ml at this point. If the level of DENA is maintained at 20 mg/kg or lower, tumor induction-time is longer, tumor growth rate is reduced, and AFP levels are of a lower magnitude. We recently started 6 animals each at levels of 1, 5, and 10 mg/kg in an attempt to determine if there is a carcinogenic threshold dose for DENA.

Other compounds which have induced hepatic cell carcinomas, have a less predictable AFP response. Tumors induced by PIP, aflatoxin, and cycasin generally have a much longer induction period (4 - 9 years), slower growth rate, and less invasive type of tumor. Consequently, laparatomies performed when the animals first become AFP positive (by immunodiffusion) have shown a wide variety of liver conditions ranging from isolated nodules to advanced tumor. Thus the DENA induced simian hepatoma represents an excellent animal model for further studies involving the relationship between AFP synthesis and carcinogenesis. The induction of tumors is quite uniform, the host has a very low spontaneous tumor incidence, and the experimental animal is phylogenetically similar to man.

Herpesvirus Saimiri (HVS)

Chemotherapy of owl monkeys with malignant lymphoma and lymphocytic leukemia induced by HVS has been continued and results suggest that cytoxan, cytosine arabinoside, vincristine and steroids and human interferon are effective in the treatment of owl monkeys with this disease. In addition several agents were examined for their effects on the multiplication of HVS *in vitro*. The multiplication of HVS *in vitro* was inhibited by cytosine arabinoside, adenine arabinoside, and tilorone, but not by rifamycin SV.

Alpha-Feto Protein Studies

With the use of the relatively insensitive double diffusion technique which allows detection of AFP in the range of 400 ng to 1 μg/ml AFP can be detected well in advance of any histological evidence of tumor - generally tumor can be predicted in an animal 6 - 7 months prior to histological evidence. In addition, in a co-operative study with Dr. McIntire the serum AFP has been quantiated using a sensitive double-antibody radioimmunoassay. This technique allows us to detect nanogram amounts of AFP - the control value for monkeys being in the range of 12 ng/ml.

The serum levels in biopsy-proven disease were scattered over a wide range of 100,000 fold variation, which is analogous to the range seen in human hepatocellular carcinoma. Monkeys with N-nitrosodiethylamine induced neoplasms had significantly higher levels of AFP than those induced with 1-nitrosopiperidine, cycasin or aflatoxin B_1. Serial determinations of AFP levels demonstrated a tendency for each monkey with liver tumor to plateau after reaching a level characteristic for that tumor.

The half-life of native serum AFP was measured under three different conditions: 1. Hepatoma serum injected IV into adult monkeys, 2. the post-partum decline in neonatal monkeys and 3. the decline in a hepatoma-bearing monkey following surgical removal of the tumor. Despite many obvious and subtle differences among these situations the results tended to corroborate a T 1/2 for AFP of 2.8 - 3.6 days. In both the neonatal and post-surgical removal of tumor there may be continued synthesis of AFP, perhaps declining in the neonatal monkeys and increasing in the monkey which develops recurrent tumor 1 month post-surgery.

Purified and ^{131}I-labeled AFP also gave a similar T 1/2 in both normal and hepatoma-bearing monkeys. This would indicate that it was possible to purify and label AFP without producing a denatured protein with a markedly different catabolism.

Synthesis of AFP was demonstrated in 7 different fresh biopsy specimens for 2 - 6 days after tumor removal. The synthetic rate measured by incorporation of ^{14}C amino acids varied but appeared linear for 2 - 6 days and where incorporation was measured during the first day it appeared to be linear. From this it would appear that in vitro synthesis during the 24 - 48 hrs following tumor removal approximated in vivo synthesis, although many other experiments using differing culture conditions are necessary before this assumption can be made. The fact that smaller amounts of tumor (20 mg) seemed to synthesize more AFP than larger amounts (50 mg) points to the importance of nutritional factors. A difficulty yet to be overcome is the counting of tumor cells; when done before the synthesis studies this destroyed 50 - 90% of the cells. Experiments are necessary to correlate the weight of wet tumor with cell number and these are in progress.

Further work on catabolic rates of AFP where there is a varied total body load and establishment of rates of cellular synthesis will allow the estimation of total body tumor cell number and the changes that take place as a result of various modalities of tumor therapy.

Diagnostic Studies

Diagnostic studies for primary hepatocellular carcinoma which may be applicable to human primary and secondary liver cancer have continued using ^{67}gallium administered IV and experimental X-ray contrast materials administered IV and via the hepatic artery. Studies to date indicate that these materials may well be useful in the diagnosis of primary and secondary liver tumors and for careful evaluation of effects of anticancer drugs on liver tumors.

Significance to Bio-medical Research and the Program of the Institute:

Tumors induced in non-human primates are likely to be more useful than the rodent tumor system now used to predict the usefulness of anticancer agents for man. In particular, the owl monkey may serve as a useful model for lymphoma and leukemia and for combination therapy with antiviral agents as well as for examination of the effects of human interferon. The rhesus induced hepatoma system appears to be an excellent model for human hepatoma both with regard to response to therapy and AFP synthesis. Potential diagnostic tests for precancerous and malignant tissue may also be discovered by use of non-human primate tumors; few such tests now are useful for man. The usefulness of immunotherapy in the treatment of precancerous conditions as well as frank neoplasia can be ascertained with the DENA induced precancerous lesions and liver tumor model. In addition the susceptibility of non-human primates to known rodent carcinogens and to possible carcinogens in man is of theoretical, practical, and economic importance. Studies of the carcinogenic potential of antitumor agents will indicate whether these compounds should or should not be used clinically in patients without neoplastic disease for organ transplantation and in treating arthritis and other diffuse collagen disorders. The susceptibility of the newborn monkey and the adult monkey to carcinogens and other foreign compounds is information that is needed for the clinician and for judging the safety of various food additives, supplements, and other compounds to which man is exposed.

Proposed Course:

1. Treatment with present carcinogens will be continued in the monkey, and it is hoped that an observation period of 10-15 years will give some basis for predicting which compounds are likely to be carcinogenic in man.

2. A continued effort will be made to assess the carcinogenic potential and long-term toxicity of procarbazine and other antitumor agents being used for treatment of non-neoplastic disease both singly and in combination with radiation.

3. Treatment of monkeys with liver tumors will be continued with various chemotherapeutic agents given i.v. and by intraarterial infusion.

4. Attempts will be made to develop primate tumor models of colon cancer and stomach cancer. Potential biological markers for various types of tumors will be investigated.

5. Studies will continue on the correlation of the production of AFP in monkeys with the time of appearance of tumors and with histopathologic changes in the liver and in the tumors.

6. More owl monkeys will be inoculated with HVS and when disease is apparent treatment with other antileukemic and antiviral agents will be undertaken. In addition, prophylactic chemotherapy will be initiated.

7. Various monkeys will be utilized for comparative disposition studies and toxicological evaluation of anticancer, antiviral,and antimicrobial agents.

8. Studies of the metabolism of the various classes of carcinogens in various species will be continued. Such studies, it is hoped, will give more insight into susceptibility or refractoriness of primates compared to rodents.

Publications:

1. Adamson, R. H., Smith, C. F., and Dalgard, D. W.: Induction of neoplasms in nonhuman primates by chemical carcinogens - correlation of serum alpha-fetoprotein and appearance of liver tumors. In Anderson, N. G., Coggin, J. H., Jr., Cole, E., and Holleman, J. W. (ed.): Embryonic and Fetal Antigens in Cancer Vol. 2 Feb. 14-16, 1972. Oak Ridge, Tenn. Oak Ridge National Laboratory, 1973, pp. 331-337.

2. Vermess, M., Adamson, R. H., Doppman, J. L., Rabson, A. S., Herdt, J. R., and McIntosh, C. L.: Intra-arterial hepatography: Experimental evaluation of a new contrast agent. Radiology 110:705-707, 1974.

3. Dalgard, D. W., McIntosh, C. L., McIntire, K. R., Correa, P., and Adamson, R. H.: Hepatic carcinogenesis and serum alpha-fetoprotein in non-human primates. In Masseyeff, R. (ed.): Alpha-Feto-Protein. Paris, France. Proceedings of the International Conference, Institut National de la Sante et de la Recherche Medicale, 1974, pp. 211-216.

4. McIntire, K. R., Adamson, R. H., Waldman, T. A., and Dalgard, D. W.: Metabolism of alpha-fetoprotein in non-human primates. In Masseyeff, R. (ed.): Alpha-Feto-Protein. Paris, France. Proceedings of the International Conference, Institut National de la Sante et de la Recherche Medicale, 1974, pp. 301-312.

Serial No. NCI-6108
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Mechanism of Action, Mechanisms of Resistance and Anti-Metastatic Activity of Anticancer Agents

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson, T. Phillip Waalkes, and David R. Makulu

Other Investigators: Susan S. Fabro, Mary K. Wolpert, Richard L. Cysyk, David G. Johns, John Krolikowski, and Sidney T. Yancey

Cooperating Units: None

Man Years:

Total:	3.0
Professional:	1.5
Others:	1.5

Project Description:

Objectives:

The knowledge of the mechanism of action of anticancer agents and why cells become resistant to them will aid in the design of new chemotherapeutic drugs and will aid in the treatment of neoplastic diseases. Specifically:

To determine the mechanism of action of various anticancer agents.

To determine the mechanism of resistance to various anticancer agents.

To design antitumor agents that will inhibit metastases or tumor growth by affecting blood vessels and fibrin cross-linking.

To design new anticancer agents utilizing knowledge obtained so that a higher cell kill can be obtained and the problem of resistance can be circumvented.

Methods Employed:

Usual biologic, pharmacologic, and biochemical techniques. Among these are tumor transplantation, harvesting of cells, tissue culture techniques, separation and analyses of whole cell DNA, RNA, and protein synthesis. In addition various enzymes are isolated, purified and the effect of various antitumor agents as inhibitors of these enzymes are studied.

Major Findings:

Effects of Antitumor Agents on DNA Polymerases from Walker 256 Carcinosarcoma

Walker 256 carcinosarcoma in rats has been a useful tumor for the evaluation of potential chemotherapeutic agents. Recently, several compounds with proven antiviral activity were also shown to inhibit the growth of Walker 256 carcinosarcoma, suggesting the possibility that an oncogenic virus, through a mechanism such as recruitment, for example, might be an essential factor for the growth of the tumor. In view of the consistent finding of an RNA-directed DNA polymerase associated with RNA tumor viruses, and in an effort to gain a better understanding of the effects of chemotherapeutic agents on this tumor, a study of the DNA polymerases of Walker 256 carcinosarcoma was made. Following extraction, separation, and purification, four distinct DNA polymerase activities were found, including an RNA-directed DNA polymerase as judged by current criteria. The ability of selected antitumor, antiviral, and anti-DNA polymerase compounds to act as inhibitors of these enzymes *in vitro* was studied. At concentrations equivalent to those producing antitumor effects *in vivo*, the compounds showed considerable variation in the degree of inhibition of the respective enzymes. None of these compounds caused more than 40% inhibition of the RNA-directed DNA polymerase which suggests that their *in vivo* antitumor properties were probably mediated through some mechanism of action other than inhibition of this enzyme.

Evaluation of the Mechanism of Therapeutic Action of Aromatic Diamidines

In spite of the growing clinical usage of aromatic diamidines, the precise mechanism of the anti-*Pneumocystis carinii* or anti-protozoa action remains obscure. Several observations suggested that these drugs may act by antagonizing folate metabolism.

We have shown that pentamidine and propamidine inhibit dihydrofolate reductase in a manner reminiscent of folate antagonists such as methotrexate and pyrimethamine. The enzyme isolated from the protozan *Trypanosoma cruzi* was found to be much more sensitive to the aromatic diamidines than mammalian tissue enzymes. It is speculated that this selective sensitivity of protozoan enzyme to aromatic diamidines may be the basis of action of these drugs.

Mechanism of Action of Monodansylcadaverine

Certain sulphonamide compounds particularly N-(5-aminopentyl)-p-toluenesulphonamide (tosylcadaverine), and N-(5-aminopentyl)-5-dimethylamino-1-napthalene sulphonamide (monodansylcadaverine) have been reported to have high inhibitory activity in the enzymatic cross-linking of fibrin. Monodansylcadaverine prevented the establishment of the plasma-cell tumor YPC-1 in mice when mixed with the tumor in concentrations of 1.5 mg/ml - 4.0 mg/ml and injected subcutaneously or intraperitoneally. Monodansylcadaverine also prevented the establishment of leukemia L-1210 in mice when mixed with the tumor in concentrations of 1.5 mg/ml - 4.0 mg/ml and given subcutaneously. Monodansylvadaverine at doses of 15.0 mg/kg and 30.0 mg/kg given as two injections on days 8 and 1 prior to YPC-1 tumor inoculation caused a reduction in tumor size, but had little or no effect on the survival time of the treated mice over the vehicular or untreated controls. These experiments indicate that monodansylcadaverine may inhibit the stabilization reaction when mixed with tumor. Since the plasma-cell tumor YPC-1 possess both clot-forming and clot-stabilizing enzymes, it is possible that the inhibition of the clot-stabilizing enzyme in the tumor provided a means to prevent stabilization of the clot. Thus, the tumor appears to have been controlled by denying it the fibrin network.

Hycanthone

The effects of hycanthone on various types of rapidly proliferating tissue, including tumor cells *in vitro* and *in vivo* and phytohemagglutinin (PHA)-stimulated lymphocytes was investigated. Hycanthone was cytotoxic to several tumor cell lines in tissue culture and exerted antitumor activity *in vivo* against Walker 256 carcinosarcoma, leukemia L-1210 and mast cell P815. Human PHA-stimulated lymphocytes incubated in the presence of hycanthone showed a 50 per cent depression in mitotic index, and a marked increase in number of chromosomal aberrations. Studies showed that hycanthone inhibits both DNA and RNA, but not protein synthesis, in human PHA-stimulated lymphocytes. Furthermore, hycanthone was found to inhibit aldehyde oxidase and this inhibition was greater than that previously reported for the N-alkylphenothiazines. These studies suggest that hycanthone may have promise as an antitumor agent, either alone or in combination with antitumor agents known to be inactivated by aldehyde oxidase.

Interferon

Mouse interferon was found to have antitumor effects against the transplantable tumor A-RCS but not against leukemia L-1210 or P388. Furthermore, human interferon was found to have antitumor effects against HVS induced lymphoma-leukemia in owl monkeys. The mechanism of this antitumor effect is not a direct cytotoxic effect since interferon *per se* is not active in tissue culture. Further studies in progress will help to define the mechanism of this agent.

Significance to Bio-medical Research and the Program of the Institute:

Knowledge of the mechanism of action of anticancer agents will lead to the design of newer drugs, hopefully, with greater cell kill and less toxicity. Studies on agents that may prevent metastases as well as the mechanism by which cells metastasize will lead to better anticancer therapy. Knowledge of the mechanism of resistance to various anticancer agents will lead to measures that minimize development of resistance or to overcome this resistance. In addition, there is the possibility that resistant cells can be converted into sensitive cells.

Proposed Course:

The mechanism of antitumor activity of several newer anticancer agents, including coralyne, maytansine, inosine dialdehyde, and NSC-141,549 will be investigated. Further studies with regard to mechanism of resistance to anticancer agents and agents that may prevent tumor metastases will also be undertaken.

Publications:

1. Sieber, S. M., Whang-Peng, J., Johns, D. G. and Adamson, R. H.: Effects of hycanthone on rapidly proliferating cells. Biochem. Pharmacol. 22: 1253-1262, 1973.

2. Yancey, S. T., and Laki, K.: Evaluation of monodansylcadaverine for effects on tumor growth. J. Nat. Cancer Inst. March 1974, in press.

3. Waalkes, T. P., Sanders, K., Smith, R. G. and Adamson, R. H.: DNA polymerases of Walker 256 carcinosarcoma. Cancer Res. 34: 385-391, 1974.

4. Hart, M. M., Reagen, R. L., and Adamson, R. H.: The effect of DDD on the ACTH-induced steroid output, histology and ultrastructure of the dog adrenal cortex. Toxicol. Appl. Pharmacol. 24: 101-113, 1974.

5. Waalkes, T. P. and Makulu, D. R.: Pharmacologic aspects of pentamidine J. Nat. Cancer Inst. Mono. 1974, in press.

This report was previously entitled, Mechanisms of Action and Mechanisms of Resistance to Anticancer Agents.

Serial No. NCI-6116
1. Lab. of Chemical Pharmacology
2. Drug Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies with Hepatic Aldehyde Oxidase, Xanthine Oxidase, and Other Drug-Metabolizing Enzymes from Mammalian Liver Cytosol

Previous Serial Number: Same

Principal Investigator: David G. Johns

Other Investigators: Mary K. Wolpert

Cooperating Units: None

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description:

Objectives:

The two molybdoflavoenzymes present in mammalian liver cytosol, xanthine oxidase and aldehyde oxidase (EC 1.2.3.1 and EC 1.2.3.2), are able to catalyze the hydroxylation of a number of N-heterocyclic compounds with activity as antitumor agents. Among substrates for these two enzymes are 6-thiopurine, 6-methylthiopurine, 3',5'-dichloromethotrexate, azathioprine and formycin B. In addition, through their ability to utilize nitro compounds as electron acceptors, these enzymes can convert carcinogens such as 4-nitroquinoline-N-oxide to their active hydroxylamino forms. The objective of this project is to characterize the range of drug and carcinogen substrate activity of these enzymes, and to develop agents with the capacity to inhibit these two enzyme activities *in vivo*.

Methods Employed:

Hepatic aldehyde oxidase is obtained from a number of mammalian species (rabbit, guinea pig, mouse and rat) and purified by ammonium sulfate fractionation followed by gel filtration, column chromatography and other standard enzyme purification methods. Activity of potential aldehyde oxidase inhibitors is determined by assaying the ability of test compounds to slow the enzyme-catalyzed conversion of

N-methylnicotinamide to its 6-pyridone, utilizing spectrophotometric assay methods. Nitroreductase activity is assayed spectrophotometrically under anaerobic conditions, using either nitrofurazone or the carcinogen 4-nitroquinoline-N-oxide as electron acceptor. The products of enzymic reduction are identified by ultraviolet and infra-red absorption spectroscopy, by thin-layer and paper chromatography, and by carbon-nitrogen-hydrogen combustion analysis. Reference compounds are prepared by chemical reduction of the parent nitro compound.

Major Findings:

A series of substituted acridine derivatives has been found with K_I-values as inhibitors of mammalian aldehyde oxidase in the range 10^{-8} to 10^{-9} M. These agents represent the most potent aldehyde oxidase inhibitors described to date. Lesser inhibitory activity has been observed with a group of pyrazolopyrimidine derivatives, with K_I-values in the range of 10^{-6} to 10^{-7} M. In studies with the aldehyde oxidase substrate 3',5'-dichloromethotrexate, it has been found that esterification of the glutamate carboxyl groups results in greatly enhanced substrate activity. In studies with nitro compounds as electron acceptors, nitrofurazone, 2-bromo-5-nitrothiazole, and the carcinogen 4-nitroquinoline-N-oxide were readily reduced by aldehyde oxidase under anaerobic conditions to the corresponding hydroxylamino compounds; a variety of other nitro compounds, including p-nitrobenzoate, did not show detectable acceptor activity. Effective electron donors for the reduction included aldehyde substrates such as butyraldehyde and benzaldehyde, and N-heterocyclic substrates such as 2-hydroxypyrimidine, 3'5'-dichloromethotrexate, and N-methylnicotinamide. NADH and NADPH failed to show detectable electron donor activity. The nitroreductase activity of aldehyde oxidase was significantly inhibited (>50%) by menadione (15 μM) and by Triton X-100 (7×10^{-4}%); in contrast to that of xanthine oxidase, however, it was not inhibited by 4-hydroxypyrazolo(3,4-d)pyrimidine (allopurinol) (5 μM). The ratios of aldehyde oxidase:xanthine oxidase nitroreductase activity in the 105,000 x g supernatant fraction of liver homogenates from several mammalian species, utilizing nitrofurazone as electron acceptor, were as follows: rabbit, 4.8:1; guinea pig, 1.4:1; and rat, 0.9:1.

Significance to Bio-Medical Research and the Program of the Institute:

Since aldehyde oxidase and xanthine oxidase both catalyze the oxidation and pharmacological inactivation of a number of N-heterocyclic antitumor agents, inhibitors for these two enzymes *in vivo* would be of both theoretical and practical interest to the chemotherapy program. With reference to the nitroreductase activity of these two enzymes, nitro compounds are widely distributed in the environment, and are readily reduced *in vivo* to hydroxyamino, nitroso, and amino derivatives. The reduced forms of certain nitro compounds are known to possess activity as carcinogens. Study of the nitroreductase activity of aldehyde oxidase and the assessment of its quantitative significance in nitro group reduction *in vivo* can thus lead to information of potential relevance to the etiology of neoplastic disease.

Proposed Course:

Compounds with significant aldehyde oxidase-inhibitory activity *in vitro* will be tested *in vivo* for their ability to slow the hydroxylation of antitumor agents subject to inactivation by this enzyme. With reference to the nitroreductase activity of aldehyde oxidase and xanthine oxidase, attempts will be made to study nitroreductase activity under conditions more nearly approximating those obtaining *in vivo*, and the effect of specific aldehyde oxidase inhibitors on nitro group reduction and on the carcinogenic activity of nitroquinoline-N-oxide in the whole animal will be carried out.

Publications:

Wolpert, M. K., Althaus, J. R. and Johns, D. G.: Nitroreductase activity of mammalian liver aldehyde oxidase. *J. Pharmacol. Exp. Ther.* 185: 202-213, 1973.

Johns, D. G., Farquhar, D., Wolpert, M. K., Chabner, B. A. and Loo, T. L.: Dialkyl esters of methotrexate and 3',5'-dichloromethotrexate: Synthesis and interaction with aldehyde oxidase and dihydrofolate reductase. *Drug Metab. Disposition* 1:580-589, 1973.

Johns, D. G., Farquhar, D., Chabner, B. A. and McCormack, J. J.: Effect of carboxyl group esterification of methotrexate on its properties as an inhibitor of dihydrofolate reductase and a substrate for aldehyde oxidase. *Biochem. Soc. Trans.* 2: in press, 1973.

Serial No. NCI-6120
1. Lab.of Chemical Pharmacology
2. Biochemical Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Kinetic Profile on Drug Action *In Vivo*

Previous Serial Number: Same

Principal Investigators: Bruce A. Chabner and Robert C. Young

Other Investigators: Charles B. Myers and Steven Rosenoff

Cooperating Units: Solid Tumor Service, Medicine Branch, NCI

Man Years:

Total: 2

Project Description:

Objectives:

In using any pharmacologic agent, certain critical information is needed in order to design an optimal schedule of drug administration. One must know:

1) The nature and kinetics of distribution and disposition of the agent.
2) Its mechanism of action.
3) The levels of parent drug or actor metabolite required for the desired effect on target tissue.

While extensive information is now accumulating with regard to pharmacokinetics and mechanism of action of the common antitumor drugs, little is known about the drug concentrations which produce toxicity and antitumor effects *in vivo*. This important consideration is the object of our research efforts. We have chosen methotrexate as a model agent for study because it is easily measured and active as the parent compound. Secondly, its mechanism of action, the inhibition of dihydrofolate reductase, with consequent block of the conversion of dUMP to dTMP, allows one to follow its action *in vivo* by determining the incorporation of ^{3}H-UdR into DNA.

We have also extended our studies to 5-fluorouracil and are in a position now to determine similar correlations between plasma and intracellular levels of F-dUMP and inhibition of thymidylate synthesis by 5-FU.

Methotrexate Studies

Methods Employed:

Methotrexate (MTX) levels were measured as previously described by the standard dihydrofolate reductase inhibition assay which allows quantitation of 10^{-9}M or greater MTX.

Major Findings:

1. Determination of MTX pharmacokinetics and time course of inhibition of ^{3}H-UdR incorporation in patients with ovarian carcinoma. Five patients with ovarian carcinoma have been studied thus far, each receiving a standard dose of 25 mg/m^2 IV. Thereafter serial *in vitro* studies of ^{3}H-UdR incorporation into DNA were performed on bone marrow, and ascites samples and the results of these incorporation studies were correlated with the plasma and ascites levels of MTX. The detailed results of this study will be described by Dr. Robert Young in the Medicine Branch Annual Report (Serial No. NCI-3420). The major research findings regarding pharmacokinetics were a three phase disappearance from plasma with a terminal 1/2 life of 12 hours to 72 hours. The longer third phase correlating with the presence of partial renal obstruction and marked myelosuppression. Preferentral accumulation of drug in ascites was observed in two patients. Efforts to define binding protein by equilibrium dialysis were unsuccessful. In addition there appeared to be no binding protein capable of reducing the cytocidal effect of MTX in tissue culture.

The bone marrow of patients with ovarian cancer recovers from inhibition by MTX at plasma levels of 10^{-8}M, or approximately the same threshold as mouse bone marrow. DNA synthesis in ascites cells was only briefly and variably inhibited by MTX despite high levels in the ascites, indicating biochemical resistance of these cells to MTX.

2. Mechanism of action of MTX rescue by leucovorin. The mechanism of leucovorin rescue following MTX is poorly understood. It appears to involve competition between the two agents in that the dose of leucovorin required for rescue is dependent on the dose of MTX administered. We have begun studies of the effect of leucovorin on plasma levels of MTX in CDF_1 mice and have found somewhat lower levels of MTX after leucovorin. These studies must be performed at higher doses of MTX and leucovorin before any firm conclusions can be drawn.

A second area of investigation is the effect of leucovorin on the depression of incorporation of ^{3}H-UdR into DNA following MTX. Our preliminary experiments with mouse bone marrow and gastro-intestinal epithelium disclosed little reversal of the depressed UdR incorporation, despite rescue by leucovorin. We will examine other possible sites of reversal, such as purine synthesis and incorporation into DNA or RNA.

3. 5-Fluorouracil Studies. The enzymatic assay for 5-FdUMP, the active metabolite of 5-FU, has been set up and has proved highly sensitive and specific. Conditions for the assay in terms of substrate (dUMP) concentration, temperature, and enzyme concentration have been defined and the assay has been used to define the intracellular conditions for suppression and recovery of DNA synthesis in mice bearing the P1534 leukemia. These studies demonstrated a characteristic recovery of UdR-incorporation into DNA at substrate/inhibition (dUMP/FdUMP) levels of 10^4 for normal tissues and 10^5 for the tumor line. Recovery of DNA synthesis appear to be related mainly to a progressive increase in dUMP levels rather than a fall in FdUMP, the levels of which decline quite slowly during their final phase of intracellular disappearance. The relationship between substrate, inhibitor, and target enzyme levels in determining final enzyme activity has been confirmed by *in vitro* studies which have duplicated the interrelationships of these variables to percent total enzyme activity observed *in vitro*. These analytical methods appear applicable to the general problem of enzyme inhibition by tight-binding inhibitors, and quantitatively show the importance of enzyme and substrate levels in determining drug effect.

In the coming year we would like to study the changes in these variables in sensitive and resistant neoplasms to document their role in determining response. Secondly we want to study the co-operative and antagonistic effects of MTX on 5-FU in terms of MTX induced changes in dUMP levels and the availability of 5-10 methylene THFA, the necessary cofactor in the binding of FdUMP to thymidylate synthetase. Finally we would like to extend this work to the study of the effect of 5-FU on human tumor and marrow cells, as we have done with MTX.

Proposed Course:

1. Complete MTX studies in man. We have performed serial analyses of ^{3}H-UdR incorporation into bone marrow and ascites tumor in 5 patients with ovarian carcinoma and in general have found a similar threshold for inhibition of bone marrow DNA synthesis in man. Tumor sensitivity has varied widely, however, and further studies are planned to obtain a clearer idea of this aspect of the work. We have noted elevated levels of MTX in ascites fluid as compared to plasma and plan to study this with regards to the presence of a folate binding protein in ascites.

2. Determine correlation of FdUMP levels and inhibition of ^{3}H-UdR incorporation following 5-FU in tumor and normal tissues of tumor-bearing mice.

3. Extend these observations to man to determine whether such correlation exists for inhibition of DNA synthesis in bone marrow and in tumor cells.

Publications:

Chabner, B. A., and Young, R. C.: Threshold methotrexate concentration for inhibition of DNA synthesis in normal and tumorous tissues. *J. Clin. Invest.*, 52: 1804, 1973.

Serial No. NCI-6121
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Purification and Properties of Carboxypeptidase G_1

Principal Investigator: Bruce A. Chabner

Other Investigators: Joseph R. Bertino, David Makulu, David G. Johns, and Robert C. Young

Cooperating Units: Department of Pharmacology, Yale University School of Medicine; New England Enzyme Center of Tufts University; Solid Tumor Service, Medicine Branch

Man Years:

Total: 1

Project Description:

Objectives:

1. To simplify and improve the purification of carboxypeptidase G_1 (CPD G_1) in order to allow large scale production of this enzyme.

2. To determine the antitumor activity of CPD G_1 in mice.

3. To determine the toxicity of CPD G_1 in mice, dogs, and monkeys.

4. To begin clinical trials of this agent if warranted by animal data.

5. To investigate prevention of methotrexate toxicity by CPD G_1.

Results:

CPD G_1, a folate-cleaving enzyme from Pseudomonas stutzeri, has been purified to homogeneity by a series of ion exchange, gel filtration, and electrofocusing steps.

Mr. Henry Blair, New England Enzyme Center, Tufts University, and Dr. Chabner from this department, have worked out a purification procedure for CPD G_1 allowing preparation of homogenous enzyme in large quantities. The method involves attachment of crude enzyme to CM-sephadex at pH 6.0 and specific elution with Na-glutamate. An improved fermentation technique has also been worked out using 10-fold increases in substrate concentration and yielding a 10-fold greater quantity of enzyme. Under contract with this section, the New England Enzyme Center has provided us with 200,000 units of highly purified CPD G_1, which will be tested shortly for clinical activity at Yale University by Dr. Joseph Bertino. Further clinical studies of this agent will await support for additional enzyme fermentation and purification.

Other research studies on CPD G_1 this year have included the following:

1. Demonstration *in vivo* that therapeutic doses of CPD G_1 have no effect on DNA synthesis in bone marrow and GI epithelium, while dramatically inhibiting both UdR and TdR incorporation in L1210 tumor cells. This effect on TdR incorporation into DNA differs strikingly from the effect of methotrexate on murine and human neoplasms, in that only UdR incorporation is suppressed initially by MTX.

2. Rabbit anti-CPD G_1 antibodies have been developed in response to CPD G_1 administration subcutaneously in Freund's adjuvant. These antibodies are capable of inactivating CPD G_1 *in vitro* and depress CPD G_1 plasma levels when administered passively to mice, but this depression appears reversible with time in that a secondary rise in plasma CPD G_1 levels is seen 6 hours after antibody administration. No antibody has been detected in dogs or mice given CPD G_1 on acute or chronic administration schedules.

3. CPD G_1 activity *in vivo* has been shown by its ability to cleave circulating methotrexate and to "rescue" animals from lethal methotrexate toxicity if given 24 hours after the antifolate. These experiments were published in *Nature* and represent the first demonstration of drug metabolism *in vivo* by passively administered enzyme.

Publications:

None

Serial No. NCI-6124
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemical and Pharmacological Studies Designed to Improve Clinical Cancer Chemotherapy: 1) Biological Markers of Malignancy; 2) Enzymatic Reactions which may be Exploited for the Development of Better Chemotherapeutic Agents

Previous Serial Number: Same

Principal Investigator: T. Phillip Waalkes

Other Investigators: Richard H. Adamson, David Makulu, Peter Sullivan and Douglass Tormey

Cooperating Units: Medical Breast Cancer Service

Man Years:

Total:	4 1/2
Professional:	2 3/4
Other:	1 3/4

Project Description:

Objectives:

1. The Development of a Biologic Markers Program. Because of the need for sensitive methods for determining not only the presence of malignant cells but also their number in any patient with cancer, a Biologic Markers Program has been initiated. The Program objective is to develop specific and sensitive techniques to detect and to measure biochemical or biological materials which are quantitative indicators of the cancer cell. Such markers are important in the evaluation of response to therapy, in determining the course of disease, and in estimating the amount of residual tumor.

2. Specific enzyme reactions involving RNA and DNA were studied from a variety of approaches. In particular, RNA dependent DNA polymerase and DNA dependent DNA polymerase in several different tissues and types of cells were evaluated as to their relationship to tumor cell growth and proliferation. Primary emphasis was placed on the separation, isolation, characterization of the DNA Polymerases of Walker-256 carcinoma, a tumor frequently used to study antitumor agents. The study of specific antitumor

drugs as enzyme inhibitors was carried out. This project has now been completed and the results published in the Feb. 1974 issue of Cancer Research.

Methods Employed:

1. Various analytical techniques are being used: (1) High-pressure liquid ion exchange chromatography in conjunction with such analytical tools and high and low resolution MS; (2) Gas-liquid chromatography (GLC); (3) Amino acid analyzers and (4) Radioimmunoassay. Contracts have been established to carry out the various types of assays, with the Oak Ridge National Laboratories for high-pressure liquid chromatography and the University of Missouri for other types of analytical procedures. These contracts complement the in-house research efforts.

2. Because of in vivo tumor inhibition by certain chemotherapeutic agents which are also anti-viral compounds, Ca-256 cells were studied for DNA polymerase activity and to detect the possible existence of a reverse transcriptase. Standard techniques with radioactive labeled compounds were used to study the in vitro kinetics of the enzymatic reactions. Purification was carried out with high speed centrifugation, DEAE chromatography, phosphocellulose chromatography, and glycerol gradient centrifugation.

Major Findings:

1. Studies are in progress to develop methods and to evaluate whether or not specific materials are elevated in the blood or urine of cancer patients. Initially, patients with advanced disease were entered into these studies. Results indicate that specific tRNA degradation products are increased in the urine of some patients with cancer. Further studies are in process to determine the extent of these elevations and the types of tumors associated with increased excretion. Polyamines also are elevated in certain cancer patients urines. Further studies regarding methodology are necessary before final conclusions can be reached. Other potential markers now in study include: CEA, α-FP, serum fucose (and other protein bound serum sugars), β-aminoisobutyric acid, Regan's isoenzyme, and hydroxyproline.

2. Several DNA polymerases were isolated including a reverse transcriptase, as judged by current criteria. These polymerases were all purified and characterized. Antitumor compounds were tested as possible inhibitors using in vitro assays. The results showed individual differences in the inhibition of the specific polymerases, but complete correlation with in vivo results was not evident.

Significance to Bio-medical Research and the Program of the Institute:

1. It is of considerable importance to cancer clinical research and to cancer patients to develop more sensitive means for determining the presence of malignant cells and to develop methods for estimating the number of these

cells present and/or remaining after therapy. The Biologic Markers Program objective is to develop methods of assay to evaluate promising materials as potential markers, and to determine their usefulness during actual clinical studies.

2. The role of reverse transcriptase has not been clarified. Its presence in a common rat tumor used frequently for assessing antitumor agents is of significance. The fact that several of the compounds which inhibit the growth of Walker-256 also inhibit specific viruses causes speculation as to the possibility that the growth of this tumor may be dependent on viral reverse transcriptase or that the tumor was originally initiated by a virus.

Proposed Course:

1. Future efforts will be focused primarily on the Biologic Markers Program. Plans are in process to increase the scope of the current program. A greater number of potential markers will be studied. Evaluations will be carried out for cancer patients before, during, and after therapy. In addition, efforts will be made to develop practical methods for determining the number of tumor cells present in patients by means of appropriate techniques.

2. No plans at present are contemplated for extending the work with polymerases of the Walker-256 tumor.

Honors and Awards: None

Publications:

1. Waalkes, T. P., Dinsmore, S., and Mrochek, J.: The presence of the nucleosides, N^2,N^2-dimethylguanosine, 1-methylinosine, and pseudouridine in the urine of cancer patients. J. Nat. Cancer Inst., 51, 271-274, 1973.

2. Gehrke, C. W., Kuo, K. C., Zumwalt, R. W., and Waalkes, T. P.: The determination of polyamines in urine by gas-liquid chromatography. In Russel, D. H. (ed.): Polyamines in Normal and Neoplastic Growth. New York, New York, Raven Press, 1973, pp. 343-353.

3. Waalkes, T. P., Sanders, K., Smith, R. G., and Adamson, R. H.: DNA polymerases of Walker 256 carcinosarcoma. Cancer Res. 34, 385-391, 1974.

4. Chang, S. Y., Lakings, D. B., Zumwalt, R. W., Gehrke, C. W.,and Waalkes, T. P.: Quantitative determination of methylated nucleosides and pseudoruridine in urine by gas-liquid chromatography. J. Lab. Clin. Med., in press.

5. Gehrke, C. W., Kuo, K. C., Zumwalt, R. W., and Waalkes, T. P.: Polyamines in human urine by an automated ion-exchange method. *J. Chromatogr.*, in press.

This report was previously entitled, Biochemical and Pharmacological Studies Designed to Improve Clinical Cancer Chemotherapy: 1) Mechanism of Action of Select Tumor Agents; 2) Biological Markers of Malignancy; 3) Enzymatic Reactions which may be Exploited for the Development of Better Chemotherapeutic Agents.

Serial No. NCI-6125
1. Lab. of Chemical Pharmacology
2. Drug Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Pharmacology of 2,6-Dimethyl-4-benzyl-4-demethylrifampicin (NSC-143414) and other Inhibitors of Viral RNA-directed DNA Polymerase

Previous Serial Number: Same

Principal Investigator: David G. Johns

Other Investigators: Mary K. Wolpert

Cooperating Units: Department of Developmental Therapeutics, M.D. Anderson Hospital and Tumor Institute, Houston, Texas

Man Years:

Total:	1.2
Professional:	0.8
Other:	0.4

Project Description:

Objectives:

2,6-Dimethyl-4-benzyl-4-demethylrifampicin (DMB; NSC-143414) inhibits viral RNA-directed DNA polymerase, delays the onset of carcinogen-induced mammary tumors in rats, and inhibits the transformation of UCl-B cells by murine leukemia virus. Because of the potential usefulness of DMB in cancer chemotherapy in man, animal pharmacology studies have been initiated. Absorption, distribution, plasma clearance, metabolism and excretion of the drug will be assessed in several animal species as an aid to the design of optimal therapeutic dosage regimens. Similar studies will be carried out with other viral RNA-directed DNA polymerase inhibitors as they become available.

Methods Employed:

DMB in biological fluids is assayed by a spectrofluorometric method or by liquid scintillation counting of drug labeled with ^{14}C in the 38 (formyl)-position of the side-chain. DMB is administered orally and intravenously to mice, rats, dogs and monkeys, and plasma, urine, cerebrospinal fluid, biliary and fecal levels of the drug are determined. Metabolites are separated from the parent drug by thin-layer chromatography, and the ratios of unaltered to metabolized drug in biological fluids ascertained. Tissue levels of the drug are determined by the combustion method followed by scintillation counting of $^{14}CO_2$, or by dissolving the tissue with the aid of solubilizing agents prior to addition to liquid scintillation counting medium.

From the data obtained, the dose-levels and frequency of administration required to give therapeutic plasma and tissue levels of the drug (5 - 50 μg/ml) are ascertained. Pharmacokinetic models are developed to permit extrapolation of the data obtained to man.

Major Findings:

DMB, either unlabeled or labeled with ^{14}C in the C-38 position, was administered to dogs at 1 or 5 mg/kg orally or intravenously. The compound was readily absorbed from the gastrointestinal tract, with plasma levels within the therapeutic range (> 5 μg/ml) being observed in the dog after oral administration of 1 mg/kg. In all three species, plasma disappearance of DMB administered by the oral, i.p., or i.v. routes was extremely slow (t 1/2: 2-4 days). In distribution studies in rats, 24 hr after an i.p. injection of ^{14}C-DMB (10 mg/kg), high tissue/plasma ratios were found in liver (6.0), pancreas (3.7), and adrenals (7.1); tissues with low levels included fat, muscle, and brain. On subcellular fractionation of rat liver homogenates, the drug was found to be concentrated in the microsomal and mitochondrial fractions. The major excretory route was biliary, yet only 4.1 ± 0.4% of the dose (mean ± SE) was found in bile within 24 hr in the rat (10 mg DMB/kg; N = 4); bile radioactivity was found on thin-layer chromatography to consist largely of unchanged DMB and the corresponding DMB-naphthoquinone, although lesser amounts of three metabolites were detectable. No circulating metabolites, other than trace amounts of the naphthoquinone form of DMB, were detected by thin-layer chromatography of serum extracts. The slow plasma disappearance of circulating DMB in all species examined may be a consequence of plasma protein binding: the DMB: albumin association constants at pH 7.4 were 2.3×10^4, 3.5×10^5, and 7.6×10^4 M^{-1} for dog, rat, and bovine plasma albumin respectively. The major significant features of the physiological disposition of DMB in the mammalian species studied are its extremely slow plasma disappearance, its tight plasma protein binding, its prolonged tissue retention, and its excretion primarily by the biliary rather than the urinary route.

Significance to Bio-medical Research and the Program of the Institute:

Of the numerous compounds which have been examined for activity as inhibitors of viral RNA-directed DNA polymerase ("reverse transcriptase"), the rifampicin series appear to offer greatest promise in terms of specificity and potency. The therapeutic trial of these and other inhibitors of the viral transformation of cells for their activity in remission maintenance in human neoplastic disease is of great current interest to the program of the Institute. The role of this research project is to provide vital whole animal pharmacology data which will be of value in designing rational dose-schedules for the use of these agents in man.

Proposed Course:

Whole-animal pharmacology studies will be continued, and sufficient further data will be collected to permit soundly based extrapolation of these pharmacological data to man. The significance of plasma protein binding in limiting the therapeutic efficacy of DMB and other members of the rifampicin group *in vivo* will also be ascertained.

If clinical pharmacological and Phase I studies with these agents are initiated, methodology developed for use in whole animal studies will be utilized for the determination of plasma and urine levels in man.

Publications:

Wolpert, M. K., Lu, K., Derr, C. J., Loo, T. L. and Johns, D. G.: Physiological disposition of 2,6-dimethyl-4-benzyl-4-demethylrifampicin in rats, mice and dogs. *Drug Metab. Disposition* 2: in press, 1974.

Serial No. NCI-6126
1. Lab. of Chemical Pharmacology
2. Biochemical Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Purification and Properties of Cytidine Deaminase

Previous Serial Number: Same

Principal Investigators: Bruce A. Chabner and David G. Johns

Other Investigators: C. Norman Coleman

Cooperating Units: Hematology and Supportive Care Branch, Plasmaphoresis Laboratory; Medicine Branch

Man Years:

Total: 2

Project Description:

Objectives:

1. To purify and characterize the enzyme cytidine deaminase found in normal and malignant leukocytes.

2. To elucidate the mechanism of enzyme regulation and its induction by cytosine arabinoside *in vivo*.

3. To explore means of utilizing the induction of this enzyme for the production of lethal pyrimidine antagonists *in vivo*.

The enzyme cytidine deaminase is known to inactivate cytosine arabinoside. Recent studies have shown that increased levels of this enzyme may be induced by ara-C in patients with acute myelocytic leukemia, and that increased deaminase is likely responsible for the development of ara-C resistance in these patients. Little is known about this enzyme in AML cells at present; thus we have undertaken detailed studies of its biochemical properties in hopes of characterizing its substrate requirements and regulation in leukemic cells.

Methods Employed:

Ara-C Project

Standard methods of biochemical analysis and protein purification were employed. Circulating leukemic cells and bone marrow from leukemic or normal donors was dispersed into 9 parts phosphate buffered saline without anticoagulant; red cells were lysed hypotonically, and white cells of the granulocyte series were collected by centrifugation and separated into mature and immature fractions by a new technique of Ficoll gradient centrifugation. The immature fraction, consisting mainly of normal blasts, promyelocytes and myelocytes, or in the case of leukemic patients, blast exclusively, was then used for further studies of the pyrimidine salvage pathway enzymes. The activities of the mature and immature fractions were also compared to determine the effect of cell maturation on enzyme levels.

Assay: Cytidine deaminase activity (CD'se) was determined by separation of product and substrate on Dowex 50 H^+ resin. An alternate method was developed for studies of 5-azacytidine and halogenated pyrimidines using a coupled assay with glutamic dehydrogenase to measure NH_4^+ production.

Results:

Both deaminase and kinase have been purified, the former 700 fold and the latter 40+ fold. Both enzymes have Km's of approximately 10^{-5} for deoxycytidine. The deaminase has greater activity for cytidine than for CdR or ara-C, while the kinase has greatest activity for CdR and ara-C and less for CR. THU is a strong inhibitor (competitive) of the deaminase, with a Ki of 10^{-7}, but has no effect on the kinase. The deaminase is much more stable in purified form than the kinase, the latter losing activity rapidly over a week's time.

Deaminase appears to increase rapidly in activity with maturation, while kinase stays relatively stable during maturation, if not declining slightly.

The kinase/deaminase ratio has been proposed as an important determinant of responsiveness to ara-C. In order to study this question in a prospective fashion, we have set up a co-operative study with the Baltimore Cancer Research Center and are determining the K/D value before, during and after therapy. Our initial efforts have defined conditions for the accurate assay of kinase activity in the presence of deaminase, using 10^{-4}M THU to inhibit the deaminase activity. The two enzymes can also be separated cleanly by DEAE ion exchange chromatography. We have determined the importance of separating cells into mature and immature fractions prior to determining the K/D ratio, which may vary as much as 50 fold in the two fractions. The K/D value in the immature cells is the only one of importance since immature cells are the only ones in active DNA synthesis and thus affected by the drug.

Proposed Studies:

1. Further purification of kinase.
2. Characterization of substrates and inhibitory activity of trinucleotides.
3. Pursue prospective study of K/D ratio in human leukemia treated with ara-C.
4. Extend investigation to other enzymes of possible significance in ara-C resistance, i.e., enzymes determining the activity of the de novo pathway, such as aspartate transcarbamylase.
5. Determine pool sizes of dCTP in leukemic cells prior to and following therapy with ara-C.

Publications:

Chabner, B. A., Evans, W. E., Coleman, C. N., Drake, J. C. and Johns, D. G.: Purification and properties of cytidine deaminase from human leukemic granulocytes. J. Clin. Invest. 53: 922, 1974.

Chabner, B. A., Drake, J. C. and Johns, D. G.: Deamination of 5-azacytidine by a human leukemia cell cytidine deaminase. Biochem. Pharmacol. 22: 2763-2766, 1973.

SUMMARY REPORT

LABORATORY OF TUMOR CELL BIOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1973 -- June 30, 1974

This Laboratory is concerned with five areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search for viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information in human leukemic cells; (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell cycle characteristics; (5) biochemical and physiological studies on leukocyte maturation *in vitro*. (This relates to a major interest of the laboratory: Does leukemia in man result from a block in leukocyte maturation?) (6) Based on new information in the literature and from studies within this Laboratory, new approaches to cancer chemotherapy are evaluated in *in vitro* and *in vivo* systems. This is the ultimate goal of the Laboratory.

During the past year a number of findings were reported by investigators from the Laboratory:

1. Demonstration that many cases of human myelogenous leukemia contain reverse transcriptase which is immunologically related to reverse transcriptase from tumorigenic subhuman primate type-C viruses.

2. Demonstration that chronic lymphocytic leukemia cells contain a reverse transcriptase-like enzyme which is not related to reverse transcriptase from known RNA tumor viruses.

3. Demonstration that the intracellular form of reverse transcriptase in type-C virus infected cells, as well as, in human leukemic cells may exist in two forms and may be associated with other cellular factors which may affect the process of reverse transcription.

4. Demonstration that placenta from rhesus monkeys contains reverse transcriptase which is immunologically related to reverse transcriptase from endogenous type-C virus from baboon placenta but not to reverse transcriptase from tumorigenic subhuman primate viruses.

5. Demonstration that cells from some patients with myelogenous leukemia contain deoxynucleotidyl transferase, an enzyme formerly thought restricted to lymphocytic cells of thymic origin.

6. Preparation of antisera to DNA polymerase I from normal lymphocytes, and the demonstration that this antisera does not inhibit other normal cellular DNA polymerases or reverse transcriptae from leukemic cells or RNA tumor viruses.

7. Purification and characterization of ribonuclease H from human leukemic cells and the demonstration that this enzyme is not associated with reverse transcriptase (as is the case for reverse transcriptase from avian myeloblastosis virus).

8. Demonstration that ribonuclease H is not associated with reverse transcriptase in mammalian type-C viruses.

9. Purification and characterization of DNA polymerase III (R-DNA polymerase) from normal and leukemic human leukocytes.

10. Development of a procedure for the rapid separation and purification of all known DNA polymerases from mammalian cells.

11. Development of a procedure for the identification of reverse transcriptase in small quantities of leukemic cells ($<$ 1 gm).

12. Purification of DNA polymerses from human leukocytes and several RNA tumor viruses for antisera preparation (in progress).

13. Development of a method of enzyme isolation and of an assay system which permits purified reverse transcriptase from type-C mammalian viruses to transcribe heteropolymeric RNA efficiently in reconstituted reactions.

14. Demonstration that under unusual reaction conditions E. Coli DNA polymerase can transcribe heteropolymeric RNA.

15. Demonstration that the endogenous DNA polymerase reaction in the microsomal fraction of human leukemic cells is both RNA-primed and RNA-directed, whereas this reaction in normal human lymphocytes is RNA-primed but DNA-directed.

16. Development of a synchronized endogenous DNA polymerase reaction in RNA-tumor viruses which may permit the sequence analysis of DNA synthesized on viral RNA.

17. Identification and characterization of ribonucleotide terminal transferase in murine type-C RNA viruses.

18. Induction of the extracellular production of type-A particles (which have previously only been reported intracellularly) and demonstration that these particles are immunologically and biologically related to type-C leukemia viruses.

19. Elucidation of the pharmacological effects of cordycepin (a post-transcriptional inhibitor) and dexamethasone (apparently a translational stimulator) on RNA tumor virus production by cells.

20. Demonstration that rifamycin SV derivatives (which were previously reported to inhibit the *in vitro* and *in vivo* activity of RNA tumor viruses after direct incubation) increased the latent period to the development of leukemia in AKR mice when administered to pregnant and to newborn mice.

21. Demonstration that the administration of cordycepin to murine leukemia virus (Rauscher) infected mice inhibited the degree of splenomegaly (50%) but did not inhibit the incidence of leukemia.

22. Demonstration and characterization of two types of leukocyte colony stimulating factor in medium conditioned by human peripheral blood leukocytes.

23. Demonstration that media conditioned by selected fibroblastic culture cells from human embryos markedly stimulate the growth and differentiation of myelogenous leukemia cells in liquid suspension culture.

24. Purification and characterization of colony stimulating factors from human lymphocytes and development of specific antisera (in progress).

25. Demonstration that the endogenous, RNA-directed DNA polymerizing complex in the microsomal fraction of human leukemic cells can be manipulated by gel chromatography and by isopycnic and velocity gradient centrifugation in a similar fashion to RNA tumor virus particles.

26. Demonstration that the genomic RNA of RNA tumor viruses are related to one another in the same manner as the phylogenetic relationship of their respective homologous host cells; this suggests that an RNA tumor virus evolves from the cellular genome.

27. Demonstration that endogenous (usually nononcogenic) RNA tumor viruses (Class I) are highly related to DNA of the progenitor cell, whereas another group (Class II) (oncogenic) are much less related to DNA from the progenitor cell.

28. Demonstration that infection of a cell with a RNA tumor virus results in marked alteration of the genome of the emerging virus.

29. Partial elucidation of the composition of RNA tumor virus genomic RNA by molecular hybridiztaion and sequence analysis.

30. Demonstration that DNA synthesized by the endogenous DNA polymerase reaction complex of human myelogenous leukemia cells has extensive sequence homology to genomic RNA from primate type-C virus; additionally, there may be sarcoma-specific sequences present in myelogenous leukemia cells.

31. Demonstration that the genomic RNA of selected RNA tumor viruses (subhuman primate; sarcoma-specific) is related to cytoplasmic DNA but not to nuclear DNA from human leukemic cells.

32. Development of microassays for the in situ detection of virus-specific nucleic acids (molecular hybridization) or proteins (antibody assays) (in progress).

Serial No. NCI-6117
1. Laboratory of Tumor Cell Biology
2. Section on Cellular Control Mechanisms
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Mechanisms Leading to Neoplastic Transformation; On Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells; On New Approaches to Cancer Chemotherapy; and on the Development of Biochemical Markers of Neoplastic Cells.

Previous Serial Number: SAME

Principal Investigator: Robert C. Gallo

Other Investigators: Major Senior Colleagues: Robert Gallagher, R. Graham Smith, Prem Sarin, David Gillespie, W. Carl Saxinger. Other collaborators and associates: Brian Lewis, Robert Mayer, Jacqueline Whang-Peng, Michael Paran, R. Mondal, Paul Gerber, Richard Adamson, Hartmut Schetters, Tony Schrecker, Robert Suhadolnik, Lawrence Loeb, George Todaro, Jeff Schlom, Umberto Torelli, Leon Dmochowski, Sol Spiegelman, Fritz Deinhardt, Robert Nowinski, Robert Ting, Ken Takemoto, Paul Peebles, William Feller, Rolf Neth, and Peter Reichard.

Cooperating Units: Laboratory of Viral Immunology, Division of Biologics Standards; Laboratory of Chemical Pharmacology, Carcinogenesis, NCI; Albert Einstein Medical Center; The Institute for Cancer Research; University of Modena; M. D. Anderson Hospital and Tumor Institute; Columbia University College of Physicians and Surgeons; McArdle Laboratories; Veterans Administration Hospital; Biotech Laboratory; National Institute of Allergy and Infectious Disease; Viral Leukemia and Lymphoma Branch, NCI; Georgetown University; Karolinska Nobel Institute, Stockholm; University of Hamburg, Molecular Hematology Department, Hamburg.

Man Years:

Total: 18
Professional: 12
Other: 6

Project Description:

Objectives

1. It is anticipated that a greater understanding of the processes involved in the molecular control of cellular growth, differentation, and carcinogenic transformation, including the pathogenesis of human neoplasias, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

3. To develop new concepts of chemotherapy and apply them to animal model systems as rapidly as possible as new information is derived from basic experimental studies.

These objectives have primarily been pursued by the following approaches:

1. Purification and properties of enzymes of nucleic acid metabolism especially DNA polymerases (DNA-dependent and RNA-dependent) of human normal and leukemic cells and of RNA oncogenic viruses to determine ultimately:

    a. The function, characteristics, and number of DNA polymerases in these cells and viruses.

    b. If RNA-dependent DNA polymerase is present in human leukemic cells and, if so, is it the same as the virion enzyme? Can it copy viral 70S RNA? Can it copy cellular messenger RNA? Does it function in gene amplification during cellular differentiation? Is its activity altered in leukemogenesis? If found, is its activity necessary for maintenance of the neoplastic cell? Can it be used as a marker of neoplastic cells? If it is not necessary for maintenance of the neoplastic state will its continued inhibition prevent some relapses of animals or patients in remission, i.e., does the inciting agent in human leukemia (? virus) remain and produce subsequent transformation?

    c. Is the viral enzyme necessary for viral replication and for initiation of transformation?

Several of the above questions have now been answered.

2. Biochemical studies on the properties of the RNA of type-C viruses and on the overall pathway of replication of these viruses. Purposes:

    a. To obtain more information on the mechanism of transcription of this RNA to DNA via reverse transcriptase.

b. To determine if diagnostic probes can be obtained, i.e., is their structure specific enough that we can use this information to find viral RNA in cells?

c. In understanding the mechanisms involved in expression of viral genes, we can plan approaches to interfere with this expression and then evaluate the overall biological effect of this interference. We particularly wish to know if viral expression is required to maintain the cell as in the neoplastic state.

3. Pursuing studies leading to an understanding of the origin of tumor viruses, how they acquire their oncogenic potential, and how they interact with cells. These studies are primarily carried out with techniques of molecular hybridization.

4. Leukocyte differentiation in vitro. The soft agar technique for investigating maturation and proliferation of normal and leukemic human bone marrow cells was recently set up following the techniques of Paran and Sachs (Paran is now in our laboratory). Attempts are made to study exogenous and endogenous (released from feeder layers of normal cells) factors which affect these processes. Attempts have been made here and in other laboratories to differentiate human leukemic blast cells with apparent success. The implications of this to understanding leukemogenesis and for potential therapeutic approaches are obvious. The mechanisms involved in the maturation process are under study.

4. Helping to elucidate the mechanisms involved in the initiation of DNA synthesis in normal and neoplastic cells and the development of potent and selective DNA polymerase inhibitors to be evaluated on fresh human acute leukemic cells and proliferating normal lymphocytes in short term culture.

5. Markers: (a) an immuno-chemical technique for finding reverse transcriptase in intact cells is now being developed. (b) Techniques for detecting viral specific nucleic acids in intact cells are also being developed.

Methods Employed

1. Human leukocytes were isolated and purified as previously described (J. Clin. Invest. 48: 105-116, 1969; Science 165: 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Biological Effects of Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334; Blood 37: 282-292, 1971).

2. DNA polymerase activities were purified and characterized as reported (Nature New Biology 240: 67-72, 1972; Proc. Natl. Acad. Sci. 69: 2879-2884, 1972; Proc. Natl. Acad. Sci. 69: 3228-3232, 1972; DNA Synthesis in vitro, Proceedings of the Second Annual Steenbock Symposium, 1972.

3. Viral reverse transcriptase was purified and studied as described (Nature 234: 194-198, 1971; J. Virol. 12: 431-439, 1973).

4. Macromolecular synthesis, viability, mitosis in leukemic and normal cells, and the effects of specific agents were evaluated as described before (J. Natl. Cancer Inst. 46: 789-795, 1971; Science 165: 400-402, 1969).

5. Product analysis of DNA synthesized by various DNA polymerases is carried out by conventional techniques including: (a) "Nearest neighbor" analysis; (b) Size estimate and nature (DNA-RNA or DNA) were made by gel electrophoresis and by glycerol gradient ultracentrifugation untreated, heat-treated, RNase and/or alkali treated.

6. *In vitro* leukopoiesis is studied by the soft agar technique developed by Paran and Sachs.

7. Induction of type-C virus from "non-producer" cells by iododeoxyuridine is carried out essentially as originally described by Rowe and colleagues. Infectious units focus formation and plaque assays for virus are carried out by conventional techniques.

8. Molecular hybridization studies are carried out by conventional and by newly evolved techniques. These include: (a) filter technique with DNA; (b) filter technique with RNA covalently attached (Proc. Natl. Acad. Sci. 70: 3219-3224, 1973); (c) Cesium sulfate gradient analyses; (d) S_1 nuclease treatment.

9. Tissue culture, virus production, cell viability estimates, cloning of cells are all carried out by standard techniques.

10. Virus quantitation, virus specific molecules, metabolism of viral RNA and proteins are studied by conventional techniques.

Major Findings

1. Our laboratory has isolated and partially purified three DNA polymerases from normal and leukemic human lymphocytes. A fourth polymerase in leukemic cells can catalyze DNA synthesis from an RNA template. This was the first finding of this type of enzyme in any animal cell. Template and other biochemical properties gave support to the possibility that this enzyme may have been viral derived. For example, the enzyme from leukemic cells will copy the genomic 70S RNA from avian RNA tumor viruses. The DNA made is complementary specifically to the template viral RNA, i.e., true reverse transcriptase. No DNA polymerase from normal human lymphocytes has this property. We later demonstrated that reverse transcriptase from human leukemic cells was immunologically closely related to reverse transcriptases from the two available type-C viruses isolated from primate tumor (gibbon ape virus and woolly monkey sarcoma virus). This study was done in part in collaboration with Dr. George Todaro of NCI.

These observations are the best available evidence that at least some human leukemic cells contain a virus specific protein namely reverse transcriptase. This does not prove that this information is causatively related to the disease, but obviously combined with evidence from animal systems these observations markedly increase this suspicion. Whether it is essential for persistence of the leukemic state remains an unanswered question.

The reciprocal immunological experiments (i.e., antibodies developed against normal human cellular DNA polymerases and to the human leukemic reverse transcriptase tested against the human cellular and viral polymerases) will be of great interest and are now in progress. We showed this past year that the major DNA polymerase of cells is not immunologically related to reverse transcriptase of known viruses or reverse transcriptase from human leukemic cells.

2. Type-C virus nucleic acid specific sequences were identified in human leukemic cells. Considerable hybridization of special cytoplasmic DNA sequences was found to the RNA of the primate type-C woolly monkey sarcoma virus.

3. Determination of the genetic relatedness of most known RNA tumor viruses. This has provided a pattern of relatedness in which it is now possible to much more critically evaluate the significance of specific types of virus-related proteins and nucleic acid in human tumor cells.

4. Development of procedures for the rapid isolation and characterization of all known DNA polymerases from normal, type-C virus-infected and leukemic human leukocytes. By such improvements in technology, it has been possible to identify and partially characterize reverse transcriptase from relatively small quantities of cells. R-DNA polymerase purified by these procedures has been completely characterized from the three types of leukocytes mentioned. These methods were used for the detection and characterization of reverse transcriptase from endogenous type-C virus particles in rhesus monkey placenta. Finally, preparative amounts of all known cellular DNA polymerases have been acquired and antisera are being developed.

5. Demonstration that cells from some patients with myelogenous leukemia contain deoxynucleotidyl transferase, an enzyme formerly thought restricted to lymphocytic cells of thymic origin.

6. Reverse transcriptase from two recently isolated primate RNA tumor viruses, Mason Pfizer Monkey virus (MPMV) and the Simian Sarcoma virus (SSV-1), had been purified by us to apparent homogeneity. The purified enzymes exhibited properties thought to be common for all viral reverse transcriptases, yet the results of these studies brought out important heterogeneity of these viral polymerases even when derived from the same species, e.g., important differences in cation requirements and molecular weight between these two primate viruses were found.

We succeeded in the development of antibodies to the primate virus reverse transcriptase and have studied the properties of these enzymes derived from cells (i.e., intracellular virus rather than released virus).

Development of a method of enzyme isolation and of an assay system which permits purified reverse transcriptase from type-C mammalian viruses to transcribe heteropolymeric RNA efficiently in reconstituted reactions.

7. Purification and characterization of ribonuclease H from human leukemic cells and the demonstration that this enzyme is not associated with reverse transcriptase (as is the case for reverse transcriptase from avian myeloblastosis virus).

   Demonstration that ribonuclease H is not associated with reverse transcriptase in mammalian type-C viruses.

8. Characterization of many of the physical properties of the sub-cellular leukemic cell particles which contains type-C virus related RNA and reverse transcriptase. Extensions of these methods may permit determination of the morphological identification and, possibly, of biological activity of these virus-like particles.

9. Demonstration that media conditioned by selected fibroblastic culture cells from human embryos markedly stimulate the growth and differentiation of myelogenous leukemia cells in liquid suspension culture.

   Purification and characterization of colony stimulating factors from human lymphocytes and development of specific antisera (in progress).

10. Elucidation of the pharmacological effects of cordycepin (a post-transcriptional inhibitor) and dexamethasone (apparently a translational stimulator) on RNA tumor virus production by cells. Using these methods in combination with assay procedures for virus-specific molecules (e.g., molecular hybridization and immune antisera) considerable insight into the life cycle of RNA tumor virus and cellular mechanisms controlling virus production has been achieved.

11. Demonstration that rifamycin SV derivatives (which were previously reported to inhibit the *in vitro* and *in vivo* activity of RNA tumor viruses after direct incubation) increased the latent period to the development of leukemia in AKR mice when administered to pregnant and to newborn mice.

    Demonstration that the administration of cordycepin to murine leukemia virus (Rauscher) infected mice inhibited the degree of splenomegaly (50%) but did not inhibit the incidence of leukemia.

The study of poly U inhibition of viral reverse transcriptase was terminated since although it was a very potent inhibitor of the viral polymerase, it lacked specificity.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, these studies are designed to obtain fundamental information on molecular and physiological control mechanisms and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

Proposed Course

As described above, some projects will terminate and others will continue to be actively pursued.

Honors and Awards:

1. "Relatedness of RNA and RNA-directed DNA Polymerase from Human Acute Myelogenous Leukemia Cells and from RNA Tumor Viruses". Presented at the Workshop on Biochemical Trends in Leukemia, Hamburg, Germany. June, 1973.

2. "Immunological Relatedness of a Human Leukemic Polymerase and Leukemia Virus Reverse Transcriptase". Presented at the 9th International Congress of Biochemistry, Stockholm, Sweden. July, 1973.

3. "Role of RNA and DNA Polymerases in Neoplastic Transformation and Cell Growth". Presented at the International Congress of Chemotherapy Athens, Greece. September, 1973.

4. "Biochemical Approaches to Detection of Viral Related Information in Human Acute Leukemic Cells". Presented at the VIth International Symposium on Comparative Research, Nagoya, Japan. September, 1973.

5. "Biochemical Approaches to Detection of Viral Related Information in Human Acute Leukemic Cells". Presented at the VIth International Symposium on Comparative Research, Nagoya, Japan. September, 1973.

6. "Characteristics and Relatedness of the DNA Polymerases and Nucleotide Sequences from Primate RNA". Presented at the Tumor Virus-Host Interaction (Sponsored by NATO), Monte Carlo, Monaco. September, 1973.

7. "Molecular Model for Human Acute Leukemia: The Virus Hot Spot Hypothesis". Presented at the Workshop on Prognostic Factors in Human Leukemia, Ulm, Germany. October, 1973.

8. "Current Topics in Metabolism". Presented at the Texas A & M University, Department of Biochemistry, College Station, Texas. December, 1973.

9. "Transfer RNA and Transfer RNA Methylases in RNA Tumor Viruses". To be presented to the International Symposium on The Biochemistry of Adenosylmethionine, Rome, Italy. May, 1974.

Publications:

1. Gallo, R. C.: Summary of Recent Observations on the Molecular Biology of RNA Tumor Viruses and Attempts at Application to Human Leukemia. The American Journal of Clinical Pathology 60: 80-87, 1973.

2. Gallo, R. C.: Some Recent Observations on the Molecular Biology of RNA Tumor Viruses and Attempts at Application to Human Leukemia. In, Polyamines in Normal and Neoplastic Growth, Russell, D. H. (ed.), (New York, Raven Press), 1973, pp. 405-414.

3. Gallo, R. C., Sarin, P. S., Wu, A. M., Sarngadharan, M. G., Reitz, M., Robert, M. S., Miller, N., Saxinger, W. C., and Gillespie, D.: On the Nature of the Nucleic Acids and RNA Dependent DNA Polymerase from RNA Tumor Viruses and Human Cells. In, Possible Episomes in Eukaryotes, (Proceedings of the IV Lepetit Colloquium), Silvestri, L. (ed.), (Amsterdam, North Holland Publishing Co., 1973), pp. 14-34.

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5. Todaro, G., and Gallo, R. C.: Human Leukemic Cell Reverse Transcriptase: Inhibition by Antibody to Primate C-type Viruses. In, Proceedings of the IV Lepetit Colloquium, entitled Episomes in Eukaryotes, Silvestri, L. (ed.), (Amsterdam, North Holland Publishing Co., 1973), pp. 117-122.

6. Gallagher, R., Levine, A., Gillespie, D., and Gallo, R. C.: Lack of Sequence Homology Between the 70S RNA of Various RNA Tumor Viruses and the DNA of Simian Virus 40 or Polyoma Virus. J. Virology 11: 1027-1029, 1973.

7. Paran, M., Gallo, R. C., Richardson, L. S., and Wu, A. M.: Adrenal Corticosteroids Enhance Production of Type-C Virus Induced by 5-Iodo-2'-Deoxyuridine from Culture Mouse Fibroblasts. Proc. Natl. Acad. Sci. U.S.A. 70: 2391-2395, 1973.

8. Bhattacharyya, J., Xuma, M., Reitz, M., Sarin, P. S., and Gallo, R. C.: Utilization of Mammalian 70S RNA by a Purified Reverse Transcriptase from Human Myelocytic Leukemic Cells. Biochem. Biophys. Res. Commun. 54: 324-334, 1973.

9. Gallagher, R. E., and Gallo, R. C.: Chromatographic Analyses of Isoaccepting tRNAs from Avian Myeloblastosis Virus. J. Virology 12: 449-457, 1973.

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11. Gallo, R. C., Miller, N. R., Saxinger, W. C., and Gillespie, D.: Primate RNA Tumor Virus-Like DNA Synthesized Endogenously by RNA-Dependent DNA Polymerase in Virus-Like Particles from Fresh Human Acute Leukemic Blood Cells. Proc. Natl. Acad. Sci. U.S.A. 70: 3219-3224, 1973.

12. Sarin, P. S., Abrell, J. W., and Gallo, R. C.: Comparison of Biochemical Characteristics of Reverse Transcriptase from Human Acute Leukemic Cells and Several RNA Tumor Viruses. In, Control of Transcription (New York, Plenum Press, 1973), pp. 345-354.

13. Gallo, R. C.: Reverse Transcriptase and Neoplasia. Biomedicine 18: 446-452, 1973.

14. Todaro, G. J., Gallo, R. C.: Immunological Relationship of DNA Polymerase from Human Acute Leukaemia Cells and Primate and Mouse Leukaemia Virus Reverse Transcriptase. Nature 244: 206-209, 1973.

15. Reitz, M. S., Smith, R. G., Roseberry, E. A., and Gallo, R. C.: DNA-Directed and RNA Primed DNA Synthesis in Microsomal and Mitochondrial Fractions of Normal Human Lymphocytes. Biochem. Biophys. Res. Comm. 57: 934-948, 1974.

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Serial No. NCI-6119
1. Laboratory of Tumor Cell Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 -- June 30, 1974

Project Title: Cytogenetic Studies: in human neoplastic, hematologic and congenital diseases; to study T and B lymphocytes to determine the origin of stem cell by using the diffusion chamber technique in mice; to study T and B proliferation, by using an in vitro chimeric system created by separation and subsequent cross-recombination of T and B lymphocytes from HL-A genotype-identical, MLC-negative siblings of opposite sex; to explore possible viral genome incorporation by using in-situ hybridization in normal and leukemic bone marrow cells; to continue to study chromosomal aberrations and the possible correlation between the development of leukemia and total body irradiation in long surviving lymphoma patients; to determine the survival of transfused lymphocytes in patients with numerous blood transfusions; to study the survival of bone marrow transplantation in humans, monkeys, and dogs; to evaluate chromosomal abnormalities caused by MTX in psoriasis treatment; to observe in vivo and in vitro effects of drugs and viral transformation.

Previous Serial Number: SAME

Principal Investigator: Jacqueline Whang-Peng

Other Investigators: Seymour Perry, Joan Bull, Douglas Tormey, John Neefe, Paul Carbone, Robert G. Graw, Jr., George Canellos, Harvey Gralnick, Susan Sieber, Richard Adamson, Ralph Johnson, Elizabeth Chu, Arnold Ratner, Geraldine Schechter, David Gillespie.

Cooperating Units: Clinical Oncology, NCI; Hematology and Supportive Care Branch, NCI; Medicine Branch, Clinical Pathology Department, Clinical Center; Radiation Branch, NCI; Laboratory of Pathology, NCI; Scientific Director, Bionetics Litton Industries; Hematology Research, Veterans Administration Hospital; Dermatologist (private practice), Bethesda, Maryland.

Man Years:

Total: 4
Professional: 3
Other: 2

Project Description: Serial No. NCI-6119

Objectives:

1. Cytogenetic studies in human neoplastic, hematological and congenital diseases.
2. To observe the *in vivo* and *in vitro* effects of drugs and viral transformation. The major effort will be the study of chromosomal aberrations correlated with the teratogenic effects of hycanthone and adriamycin.
3. To continue the study of the possible correlation between development of chromosomal aberrations and subsequent malignancies in long term lymphoma patients who have received total body irradiation.
4. To continue the study of the survival of spontaneously dividing or PHA-responsive lymphocytes in patients receiving several units of blood from the opposite sex.
5. To continue the study of chromosomal aberrations caused by methotrexate in psoriasis treatment.
6. To study bone marrow grafting in the human, monkey and dog using various techniques.
7. To study T and B lymphocytes and determine their relationship to the stem cell by using diffusion chamber culture technique in mice.
8. To study the nature of T and B lymphocytes in vitro by employing mixed cell cultures to produce a chimeric system and subsequent cross-recombination of T and B cells from HLA genotype identical, MLC negative siblings of opposite sex.
9. To study in situ hybridization of normal and leukemic marrow cells to explore the possibility of viral genome incorporation.
10. To perform cytogenetic studies of peripheral blood, breast tissue and effusions in order to correlate chromosomal findings with pathological staging, treatment, and prognosis.

Major findings:

1. Through the use of the special Giemsa banding techniques it was determined that the Ph^1 chromosome is formed by a translocation of a piece of the number 22 chromosome to the long arm of a number 9 chromosome T (9;22)(q 34;q 12).
2. Karyotype analysis of mixed T and B lymphocyte cultures showed that stimulating allo-antigens are located predominately on the B cells, but only T cells recognize these allo-antigens and respond to them by proliferating. However, the presence of monocytes is required for this T/B cell interaction, although their precise role is yet to be determined.
3. Preliminary findings using diffusion chamber culture techniques indicate that the B lymphocytes may be the source of stem cells.

Honors and Awards

Editorial Board of Blood, 1973-1974

Publications:

1. Sieber, S. M., Whang-Peng, J., Johns, D. G., and Adamson, R. H.: Effects of Hycanthone on Rapidly Proliferating Cells. Biochemical Pharmacology Vol. 22, No. 11, 1 June 1973, 1253-1262.

2. O'Connell, M. J., Ramsey, H. E., Whang-Peng, J., and Wiernik, P. H.: Testicular Feminization Syndrome in Three Sibs: Emphasis on Gonadal Neoplasia. The American Journal of the Medical Sciences 265(4): 321-333, 1973.

3. Whang-Peng, J., Knutsen, T. A., and Lee, E. C.: Brief Communication: Dicentric Ph^1 chromosome. JNCI 51: 2009-2012, December, 1973.

4. Whang-Peng, J., Lee, E. C., and Knutsen, T. A.: The Genesis of the Ph^1 chromosome. JNCI 52, April, 1974.

5. Whang-Peng, J., Gralnick, H. R., Johnson, R. E., Lee, E. C., and Lear, A.: Chronic Granulocytic Leukemia (CGL) During the Course of Chronic Lymphocytic Leukemia (CLL): Correlation of Blood, Marrow and Spleen Morphology and Cytogenetics. Blood 43: 333-339, 1974.

SUMMARY REPORT

LABORATORY OF MOLECULAR PHARMACOLOGY

DIVISION OF CANCER TREATMENT

July 1, 1973 -- June 30, 1974

Work during the past year has focused on DNA damage, repair and replication in cultured L1210 cells. The work was aided by a new technique, developed in this laboratory last year, that gives a highly sensitive measure of DNA single-strand breakage in intact cells. The procedure, which involves the alkaline elution of DNA from cells lysed on filters, was studied in further detail in order to optimize the sensitivity of the technique and to clarify what is actually being measured. The rate of elution of DNA under optimized conditions was found to depend primarily on single-strand length. This was deduced from measurements on cells that had received various doses of X-ray to produce known extents of single-strand breakage randomly distributed in the DNA. The technique was easily able to detect the effect of 50 rad, and is therefore the most sensitive known biochemical measure of X-ray damage in cells. The frequency of breaks produced by such low doses is of the order of one per 10^{10} daltons of DNA.

A variety of chemotherapeutic agents were examined for their ability to produce DNA strand breakage in L1210 cells. Of 20 agents tested, 14 produced measurable strand breakage. In most of these, the extent of breakage was relatively low, not greater than the effect of 150 rad X-ray. Of the agents tested, an exceptionally high degree of strand breakage was produced by bleomycin. The bleomycin effect, however, was distinctly non-random, as if part of the DNA is severely degraded whereas the remainder is relatively undamaged. This may reflect the strong dependance of bleomycin cytotoxicity on phase of cell cycle. Distinct changes in DNA elution were seen within 30 min of bleomycin treatment. After removal of bleomycin, there is a limited repair of DNA that occurs gradually over a period of several hours.

The same 20 agents were examined for possible inhibition of the rejoining of X-ray-induced DNA single-strand breaks in L1210 cells. The only agents active in this regard were BCNU, CCNU and their isocyanate decomposition products. The latter products were, in fact, much more active than the parent compounds. The effect was not secondary to general cell disruption since RNA synthesis was not much inhibited under the conditions used.

DNA repair after UV irradiation of cells involves enzymatic scission of DNA strands near the sites of UV damage, followed by repair synthesis and finally resealing of strand ends. Of the various aspects of this repair sequence that have been studied, the breakage and resealing events have been the most difficult to measure, because the frequency of breaks that exist at any one time is extremely low. The alkaline elution assay was found to be sensitive enough to measure this low level of breaks. The appearance and resealing of breaks after UV was observed in HeLa cells and normal human fibroblasts. Xeroderma fibroblasts, as expected, exhibited a greatly reduced rate of DNA scission. The kinetics of these events are now being studied, and testing of various chemotherapeutic agents has been begun to see whether any of them interfere with these repair processes.

DNA strand elongation during replication in L1210 cells was studied to form a basis for observation of drug effects. The time-course of the sequence of chain growth from the stage of Okazaki fragments to the formation of complete replicons was observed by alkaline sucrose gradient sedimentation. There appeared to be two points of discontinunity in the sequence: at the joining of Okazaki fragments and at the joining of replicons. The kinetics of the latter process was measured by alkaline elution, which allows observation of the later stages when strands of extreme length are formed.

Serial Nos. 1st NCI-6104
2nd NCI-6112
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effects of Chemotherapeutic Agents on DNA

Previous Serial Number: Same

Principal Investigators: Kurt W. Kohn, H. E. Kann, Jr., C. A. Friedman, A. Fornace, and Z. Iqbal

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	8
Professional:	4.5
Other:	3.5

Project Description:

Objectives:

To determine the mechanisms of interaction of chemotherapeutic drugs with DNA, and the consequences of such interactions on DNA structure and function.

Methods Employed:

Growth of L1210 cells in suspension culture; quantitative determination of colony-forming ability by cloning in soft agar; incorporation of radioactive precursors; X-irradiation of cells; special procedures for analysis of state of DNA in cells, described herein; preparative ultracentrifugation in density gradients.

Major Findings:

1) Alkaline elution analysis of DNA from mammalian cells.

This technique, which we developed last year, was studied further in order to determine the basis for the differential DNA elution that is observed. In this procedure, cells are lysed on filters by 0.2% Sarkosyl, 2M NaCl, 0.02M EDTA, pH 10 and then slowly eluted with tetrapropylammonium hydroxide - EDTA, typically at pH 12.1-12.8. The elution flow is regulated by means of a peristaltic pump, and fractions are collected at regular intervals. Various factors which affect the elution were examined in detail. The results indicated that the completed DNA of normal cells is too large to elute at an appreciable rate, but that single-strand breaks slowly accumulate with time in alkali and gradually accelerate the elution rate. The magnitude of this acceleration depends on pH and is presumably due to alkaline hydrolysis of DNA chains. The possibility that this is due to the presence of alkali-sensitive RNA segments in the DNA chain was excluded by the finding that ribonuclease treatment does not consistantly increase the elution by alkali.

The dependence of elution rate on single-strand length was determined by examining the effects of X-ray delivered to intact L1210 cells. The effects of X-ray doses from 50 to 400 rad were easily measurable. Over this range, the initial rate of elution was first-order with time, and the apparent rate constant was approximately proportional to dose. The apparent target size was about $2x10^9$ daltons, which corresponds to single strands of the order of 2 mm in contour length. Thus alkaline elution differentiates extremely long DNA strands on the basis of a length-dependant elution rate. This rate was found to be independent of pump speed and cell number, and was insensitive to the pH of elution in the range of pH 11.8-12.8. At lower pH, however, elution was reduced, and some resolution of strands of smaller length can be obtained.

2) DNA chain growth during replication in L1210 cells.

Last year we reported a kinetic study of the formation of multi-replicon DNA segments using the alkaline elution technique. We have now correlated these findings with a detailed study of chain growth using alkaline sucrose gradient sedimentation. Conditions of centrifugation were found that avoid artifactual trapping or degradation of the DNA. The chain growth sequence was examined from the shortest obtainable pulse-labeing times to the formation of extremely long DNA segments that have very rapid and anomalous sedimentation. The findings indicate that DNA is initially synthesized at least in part as small, short-lived fragments that sediment at 4-6S and correspond to "Okazaki pieces." These small segments join rapidly to form strands of intermediate length, sedimenting at 26S. Further strand elongation from 26S to 50S then occurs gradually over a period of about 1 hour. The 50S segments correspond in size to the completed replicons observed by autoradiography. These segments then join discontinuously to form the extremely long DNA strands of the anomalously sedimenting DNA aggregate. The kinetics of this last step correlates with the formation of non-elutable DNA as measured by the alkaline elution procedure. The alkaline elution procedure therefore measures the synthesis of multi-replicon segments of DNA.

3) Survey of agents for production of DNA strand breaks and for inhibition of rejoining of X-ray-induced strand breaks in L1210 cells.

Twenty agents were examined for ability to produce DNA strand breaks and for ability to inhibit rejoining of strand breaks produced by X-ray. Measurement of DNA strand breakage was carried out by the alkaline elution technique.

Strand breaks directly produced by drugs were measured after 30-60 min exposure of L1210 cells to drug in suspension culture. Effects on rejoining were measured by adding drug 10 min prior to irradiation of cells with 1,000 rad and then allowing 30 min for repair. The measurements of direct strand breakage and of inhibition of rejoining were carried out together, so that direct drug-induced DNA breakage could be distinguished from inhibited rejoining of X-ray-induced breaks. Of the wide variety of agents tested, only 6 out of 20 failed to produce detectable changes in DNA elution within 30 min. The changes in most cases were small, comparable to the effects of 100 rad or less. Bleomycin was exceptional in that it produced greater increases in DNA elution than was seen with the other drugs tested. The smaller changes produced by most agents appear to be an early measure of cell damage. Inhibition of rejoining after X-ray, on the other hand, was an usual finding and occurred to a measurable degree only in the case of BCNU, CCNU and their corresponding isocyanates.

4) DNA single-strand breakage in L1210 cells treated with bleomycin.

Although bleomycin is known to produce DNA breaks in purified systems, the results in intact cells have been conflicting. We examined the effects of bleomycin on DNA in L1210 cells using the alkaline elution technique. In order to avoid erroneous interpretations due to the tendancy of bleomycin to adhere to surfaces and to break DNA after cell lysis, it was necessary to wash cells free of the drug by centrifugation prior to cell filtration and lysis. The possibility of drug effects occurring during the analytic procedures was monitored by adding H^3-labeled control cells to drug-treated C^{14}-labeled cells at the end of the drug incubation period. When the cells were adequately washed prior to filtration, only the DNA of the drug-treated cells was affected, thus showing that the drug did indeed act on intact cells.

The effects produced on DNA elution indicated that only a part of the DNA was broken while the remainder was not. This was inferred from the shapes of the elution profiles, compared with those obtained after X-irradiation of cells. X-ray produces single-strand DNA breaks randomly in all of the cell DNA. By comparing the elution patterns of DNA from X-irradiated *versus* bleomycin-treated cells, it was concluded that the effect of bleomycin was non-random. The results were as if bleomycin treatment degraded part of the DNA but left the remainder intact. A likely interpretation is that cells differ in regard to sensitivity to the drug, possibly due to drug-uptake differences dependent on cell cycle. The alkaline elution procedure provides a convenient measure of the effect of bleomycin on cells.

Bleomycin-induced DNA damage in L1210 cells was found to be partially repaired over a period of 4 hours of incubation after washing cells free of drug.

Bleomycin has been reported to inhibit the repair of DNA after X-irradiation. We find, however, that in L1210 cells the apparent reduction in DNA strand rejoining after X-ray can be entirely accounted for by the direct production of breaks by bleomycin. We conclude that in L1210 cells, bleomycin does not inhibit repair of DNA breaks produced by X-ray.

5) Effects of nitrosoureas and isocyanates on DNA repair.

For some time inhibitors of DNA repair have been sought as possible adjuncts to chemotherapy with alkylating agents. A compound having such activity is 2-chloroethyl isocyanate, one of the breakdown products in the decomposition of 1,2-bis(2-chloroethyl)-1-nitrosourea (BCNU). The idea that 2-chloroethyl isocyanate (CIC) might interfere with the activity of enzymes involved in DNA repair came from earlier studies of nitrosourea effects on RNA metabolism. In those studies CIC, which binds to proteins but not to nucleic acids, was shown to shut off both the synthesis and the processing of RNA. These effects were tentatively attributed to inhibition of certain enzymes that act on nucleic acids; and the possibility of inhibition of other nucleic-acid-binding enzymes (e.g. repair enzymes) was entertained.

Repair of X-ray-induced single-strand breaks in DNA was estimated from the rate of alkaline elution of DNA from cells allowed to repair for varying periods of time after exposure to X-irradiation. CIC inhibited strand rejoining; BCNU inhibited, but to a lesser degree. The compound 2-chloroethylamine, which forms by hydrolysis and decarboxylation of CIC, did not affect repair. The isocyanate breakdown product of CCNU inhibited comparably with CIC. These findings were confirmed in separate experiments using alkaline sucrose gradients. A number of inhibitors of macromolecular syntheses did not affect repair in this test system; these included hydroxyurea, Ara C, Actinomycin D, and cycloheximide. From this it was clear that the CIC effect was not one commonly seen in association with metabolic inhibitions. Accordingly, additional studies were begun to see whether other steps in the repair process were affected.

Following damage to DNA from ultraviolet light the initial step in repair is enzymatic strand breakage near the region of damage. To assess the effect of CIC on this step in repair human cells were used (HeLa; normal human fibroblasts; fibroblasts from patients with xeroderma pigmentosum -- thought to lack the enzyme required for nicking DNA in the 1st step of excision repair).

6) DNA single-strand damage and repair after UV-irradiation of cells.

Of the various aspects of UV damage and repair of DNA in cells, the formation and resealing of DNA strand breaks, which has been inferred on the basis of other studies, has been the most difficult to study directly. This is because the number of breaks that exist at any one time is so small that the resulting single-strand segments are too long to be easily measured by the techniques in common use. We were able to measure this process using the alkaline elution technique. We find that in normal human fibroblasts and in HeLa cells, DNA breaks appear and reach a maximum within a few minutes after UV irradiation. The rejoining of these breaks occurs at a rate which depends on UV dose and which may extend over a period of several hours. The line of xeroderma fibroblasts was found to be defective in that breaks appeared much more slowly than in normal cells and were not repaired. CIC did not affect the rate of strand breakage after UV in HeLa cells, in normal human fibroblasts, or in L1210 cells.

Preliminary experiments were carried out with the alkaline elution technique to follow repair after exposure to BCNU. Strand breakage occurred soon after exposure of cells to BCNU; rejoining was largely completed within 18 hours.

7) Repair of X-ray damage in Progeria cells.

It has recently been reported that fibroblasts from patients with Progeria are defective in the rejoining of DNA breaks produced by X-ray. We examined cells from two progeria patients. Under the conditions of our assay, the repair in progeria cells was normal.

Proposed Course:

1) Study the effects of transient inhibition of DNA synthesis by various agents on the course of DNA replication in cells. Alkaline elution, alkaline sedimentation and isopycnic centrifugation will be used to detect effects on DNA strand growth and joining during recovery from replication block. These studies are aimed at understanding the mechanism of cell killing by DNA synthesis inhibitors, especially in the way they are employed in the overdose-rescue strategem of chemotherapy.

2) Examine diploid and transformed cells for differences in metabolic responses to various inhibitors, in order to determine whether differences analogous to those produced by serum deprivation can be elicited. Then determine whether under such conditions of metabolic stress transformed cells may become more vulnerable than diploid cells to various chemotherapeutic agents. Cytotoxicity will be guaged by cytologic criteria of viability and by alkaline elution measurement of DNA integrity. The objective is to generate potential stragems for combination chemotherapy.

3) Measure the course of DNA breakage and rejoining following treatment of various cell types with X-ray, ultraviolet light, bleomycin, alkylating agents and nitrosoureas. The objective is to determine whether any differences can be discerned between normal and neoplastic cells in their reponses and abilities to recover after treatment with these agents.

4) Continue the survey for inhibitors of DNA repair at the endonuclease and ligase steps.

Publications:

1) Kohn, K. W. and Ewig, R. A. G. Alkaline elution analysis, a new approach

to the study of DNA single strand interruptions in cells. Cancer Research 33: 1849-1853 (1973).

2) K. W. Kohn, Snyder, A. L. and Kann, H. E. Size distributions of high molecular weight RNA synthesized by L1210 cells. Effects of DNA-reactive drugs. Biochim. Biophys. Acta. 324: 93-109, 1973.

3) Kann, H. E., Kohn, K. W. and Martin, J. K.: Inhibition of DNA repair by the 1,3-bis(2-chloroethyl)-1-nitrosourea breakdown product, 2-chloroethyl isocyanate. Cancer Research 34: 398-402, 1974.

4) Kohn, K. W. Anthramycin. In Corcoran and Hahn (e.d.): Antibiotics. Springer-Verlag (in press).

5) Kann, H. E., Kohn, K. W., Widerlite, L. and Gullion, D. Effects of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and related compounds on nuclear RNA metabolism. Cancer Research (in press).

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SUMMARY REPORT

ASSOCIATE DIRECTOR FOR MEDICAL ONCOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1973 - June 30, 1974

The Medical Oncology Area has major responsibilities for clinical investigations involving new chemotherapeutic drug development, combination chemotherapy, and combined modality therapies for patients with acute leukemia, malignant lymphomas, lung, breast, and ovarian cancer. Emphasis has been placed on combined modality approaches for curative therapy potential by developing and enforcing collaborative efforts with surgery, radiation and immunological colleagues intramurally at the Clinical Center and also extramurally through national organ site programs. In combined modality approaches the Medical Oncology Area of the Division of Cancer Treatment plays a major role in lung, breast, and ovarian cancer organ directed programs. For the future we anticipate involving ourselves in prostate cancer, pediatric solid tumors, and if possible, pancreatic and gastrointestinal cancers. In addition to organ site-oriented studies, the Medical Oncology Area carries on laboratory and clinical research in protected environment studies, white cell and platelet transfusions, cellular kinetics, pharmacology, basic virology and *in vitro* marrow culture techniques, all designed to provide basic knowledge or improve therapeutic efficacy.

Program Accomplishments

The broad objectives of our clinical and laboratory studies are to apply basic biomedical research findings to problems in clinical cancer. The following items highlight some of the accomplishments of our investigators.

Preclinical Investigations

Viral Studies - IXX

Dr. Arthur Levine (Section on Infectious Diseases, Pediatric Oncology Branch) has continued his investigations on DNA oncogenic viruses. Adenovirus 2-SV40 hybrid viruses have been isolated which contain different, but overlapping portions of the SV40 genome. The larger the fragment of SV40 DNA in the hybrid genome, the more species of SV40 RNA and the more SV40 antigens are induced in infected cells. This information has been used to provide a genetic map of the early region of the SV40 genome, the region associated with cell transformation. This is the first genetic map of an animal tumor virus, and may be useful in determining which SV40 genes are necessary and sufficient to initiate and maintain the transformed state.

These hybrid viruses have also been useful in studying the details of transcription regulations in SV40, and the results suggest that this animal virus shares many common regulatory features with phage. Also, the nondefective hybrids are a model for integration: The SV40 insertion in the Adenovirus DNA is analogous to the insertion of any viral DNA into an animal chromosome. These studies have indicated that when SV40 DNA is integrated (linear) rather than in its native free (circular) form, certain genes which are responsible for formation of mature virus are not expressed. This observation may provide insight into why viruses sometimes replicate in cells and kill them, and why they sometimes remain "latent" and transform them.

Marrow Culture - IXX

Human marrow cells can be induced to produce granulocyte-macrophage colonies *in vitro* using a semi-solid media and a colony stimulating factor. During this past year studies of normal marrows have demonstrated a physiologic difference between male and female marrows, impaired growth of marrows from patients with Hodgkin's disease, lymphomas, and breast cancer with bone marrow tumor involvement. Attempts were made to study the effect of intensive combination therapy on the growth kinetics, both acutely and long-term. With the use of 6 months of MOPP therapy, long-term remission patients demonstrated no persistent abnormality. Investigations of marrow and peripheral growth kinetics of granulocyte committed stem cells may allow for better scheduling of the chemotherapeutic drugs.

New Drug Development - 2AX

Limiting toxicity of the important class of nitrosourea compounds is bone marrow. Experimental studies with aminoglucose moieties on the N3 position have clearly indicated that the intrinsic anti-tumor effect and the bone marrow toxicity can be separated. 3-(Tetra-acetyl-glucopyranose-2-yl)-1-(2-chlorethyl)-1-nitrosourea, GCNU (NSC 1114460) and d-glucopyranose,2-3[(2-chloroethyl) nitrosoamino] carbonyl[amino]-2-deoxy, DCNU (NSC 178248) have been synthesized and tested for experimental anti-tumor activity and bone marrow toxicity. DCNU looks particularly promising because of its greater activity in L1210 (including cures) and its water solubility.

Cell Kinetics - 4XX

The relative effects of drugs against tumor cells and host tissues determines the therapeutic index of a particular agent. Studies in the Section of Cell Kinetics (Dr. Robert Young, Head) have employed test systems examining the relative effect on host tissues and tumor DNA synthesis following exposure to chemotherapeutic agents. The data indicate that exploitable differences occur particularly in the recovery of DNA synthesis between tumor and normal tissues. The drugs studied include BCNU (NSC 409962), cyclophosphamide, methyl-CCNU, ARA-C, and Adriamycin. Preliminary studies are being done in patients with ovarian cancer and ascites and cutaneous metastases with melanoma.

At the NCI-VA Medical Oncology Branch several experimental tumors were under kinetic study using standard H^3-TdR and in vitro agar growth. Perturbations after chemotherapy were studied. In vivo data was obtained in bronchogenic and head and neck cancers. A comprehensive analysis of H^3-TdR disposition in man was done.

Phase I Studies - 4X1

During the past year Phase I trials were performed using thalicarpine (NSC 68075), Gallium nitrate (NSC 15200), ICRF-159, epipodophyllotoxin ethylidene glucoside (VP-16), and thalicarpine. Pharmacologic disposition studies were done with 67-Gallium nitrate, thalicarpine, epipodophyllotoxin-thenylidene glucoside (VM-26), VP-16, and oral absorption of methotrexate. Additional clinical studies were done with combination of CCNU, caffeine and chlorpromazine following initial preclinical studies indicating enhancement of the therapeutic action of CCNU.

Phase II Trials - 5XX

Streptozotocin (NSC 85998) was administered to 106 patients. Activity was demonstrated in patients with Hodgkin's disease, lymphocytic lymphomas and islet cell tumors. A trial with oral methyl-CCNU in 28 patients with malignant melanoma revealed six responses (21%). Hexamethylmelamine (NSC 13875) was used in 10 patients with advanced previously treated ovarian cancer and two responses were reported. Adriamycin (NSC 123127) administration resulted in responses in breast, sarcoma and lymphoma patients. Preliminary trials of combinations of Adriamycin and dibromodulcitol demonstrated responses in patients with breast cancer. New combinations were developed in ovarian and diffuse histiocytic lymphoma (See Phase II reports by disease).

Phase III and Phase IV (Combined Modality Trials) - 6X1 and 7XX

Disease-oriented intensive trials were conducted in lung, breast, ovarian, Hodgkin's and non-Hodgkin's lymphoma, prostate and acute lymphocytic leukemia patients. Working with Dr. Anthony Kontaratos (General Physical Science Administrator, DCT, NCI), and Dr. Stephen Carter (Associate Director, Cancer Therapy Evaluation, DCT, NCI), a generalized strategy for chemotherapy of solid tumors was developed. Subsequently, specific plans were developed for breast and ovarian cancer. Phase IV trials now apply to combined modality studies with optimal integrated therapeutic approaches with surgery, chemotherapy and radiotherapy. Some of the major efforts are listed below:

Acute leukemia: A major finding reported this past year indicated that a leukemia antigen could be demonstrated on human leukemia cells that was shared with Rauscher leukemia and RAJI cells - a Burkitt tumor cell line. Preliminary immunotherapy-chemotherapy trials using RAJI cells were begun. On NCI protocol 72-1, 62 patients with ALL after consolidation received BCG plus methotrexate or methotrexate alone. Despite evidence of improved cellular reactivity to "tumor cells" with BCG, no demonstrable differences in the remission duration of the two treatment groups were seen. A randomized trial comparing intrathecal methotrexate with or without 2400 rads to the cranium demonstrated a significantly longer (234+ days) remission duration in the group receiving methotrexate as compared to the radiated group (125 days). Methotrexate administration using the Ommaya reservoir and tied into pharmacologic measurements is being explored.

Pediatric solid tumors: Under the current plans, emphasis will be placed on developing combined modality trials in patients with pediatric tumors. Patients with neuroblastoma, sarcomas, and lymphomas are being put on new protocols developed as part of a new comprehensive clinical pediatric program. Curative activity in Burkitt's tumor continues to be demonstrated for chemotherapy regimens while additional immunotherapy with BCG fails to add to the remission duration. A major contribution of the Makerere program under Dr. Charles Olweny was the remarkable effects of Adriamycin (10/13 responses, two complete).

Hodgkin's disease and malignant lymphoma: Complete remissions occur with MOPP therapy in 80% of patients with Hodgkin's disease treated. The median duration is approximately 48 months and the estimated five year survival for complete responders is 80%. Remission maintenance regimens with continued MOPP or BCNU failed to add to the response of six months MOPP.

Non-Hodgkin's lymphoma represents a new major thrust. In studies on the Medicine Branch, the values of defining subgroups by histological variants and accurate staging have been confirmed. Combination chemotherapy programs have produced 45% complete responders in 80 patients recently reported with a median duration of 3-1/2 years. Prospective trials with new combination chemotherapy protocols employing Adriamycin and Bleomycin with Cytoxan, vincristine and prednisone are being tried to improve the proportion of complete responders and the remission duration. Of interest was the finding that total body radiation and combination chemotherapy with Cytoxan, vincristine and prednisone produced comparable results. Other prospective protocols are planned to incorporate radiotherapy into the clinical trials.

Lung cancer: Multi-modality trials are being initiated through the Lung Cancer Working Party and the NCI-VA Medical Oncology Branch employing radiation, surgery and chemotherapy. Many obstacles exist to developing effective therapy of lung cancer but a major limitation is the lack of effective single agents and relatively poor combination chemotherapy results in all cell types except small cell carcinoma. In this latter group a combination of Cytoxan, methotrexate and CCNU has a 57% response rate and a 30% prolongation of survival. Current approaches include more aggressive application of radiotherapy (cranial and abdominal radiation) combined with chemotherapy. In addition to the chemotherapy program, major efforts are underway to study cell kinetics, staging procedures, the value of protected environments, morphologic studies and quantitative evaluation of tumor response.

Breast Cancer

A variety of combination chemotherapy programs can now be shown to produce C+PR of 50 to 60%. The regimen started about three years ago in the Medicine Branch consisting of fluorouracil, Cytoxan, methotrexate and Prednisone and has produced 22 responders in 33 patients treated. This has served as a basis for several combined modality trials (surgery or oophorectomy plus chemotherapy trials) and as a standard for new combination regimens.

The Medical Breast Cancer Service has blossomed into an active clinical service with over 2500 patient visits this fiscal year. Combined medical-surgical programs have been initiated in primary and first recurrence disease protocols. Strongly associated with the patient protocols are studies designed to evaluate immunochemical and biochemical markers of disease activity. Using one of several markers about 65% postoperative node positive patients have some elevated value as do 97% of patients with metastatic disease. Correlations with treatment results are being made.

Adriamycin has been incorporated into a combination protocol attempting to improve the therapeutic results of metastatic disease patients. Major efforts are expanded to include our Division of Cancer Treatment contractors into the overall strategy with sharing of program questions, cross fertilization of information and results. All the facets of breast cancer from the primary treatment efforts, combined modality primary treatment efforts, first recurrence protocols and metastatic disease studies are conducted through the Associate Director for Medical Oncology and the Breast Cancer Task Force Treatment Committee. Specific hormone inhibitors and estrophile receptor measurements can now be added to the chemotherapeutic regimens.

Preliminary results with surgery and chemotherapy trials already are indicating a marked effect on disease recurrence. A major biological question will be answered by these studies since programs with a wide range of response rates in advanced disease patients are being tried as adjuvant regimens. We will be able to estimate what the relative response rate in advanced disease can be translated to the adjuvant situation.

Ovarian Cancer

Ovarian cancer is the fourth most common killer in women. As with breast cancer, chemotherapy plays an important role in therapy because the clinical disease very often can not be cured by local therapeutic modalities and several agents have been shown to produce significant antitumor effects. A strategic plan for combined modality approaches has been developed and serves as the basis for therapeutic trials. Intramural efforts include a reported trial comparing L-phenylalanine mustard (L-PAM) to high dose cyclophosphamide and the current trial comparing L-PAM versus a combination of hexylmethylmelamine, Cytoxan, fluorouracil and methotrexate. Planned trials include Stage I and II patients using chemotherapy, surgery and radiotherapy. The Medicine Branch has made significant advances in staging of patients using laparotomy, radioisotope scans and ultrasound recordings. Their studies have demonstrated that clinical disease extent, particularly in the subdiaphragmatic region, may account for failure following apparent adequate radiotherapy. Contract studies involving controlled Phase II trials are underway defining the activity and role of individual agents as a preliminary to developing effective combination trials.

Laminar Flow Project

A new six bed laminar flow-intensive care ward has been built and will open next year, programmed to enlarge the testing of the units in a variety of other diseases including pediatric tumors and solid tumors. Projects supported by contract are already underway.

Blood Component Transfusion Therapy

Blood component therapy provides a major resource that enhances our ability to improve the therapeutic efficacy of current chemotherapeutic agents pushed to their toxic limits. Efforts to provide more HL-A matched donor-recipient pairs resulted in establishing a contract support activity situated close to the Clinical Center. Through the University of California at Los Angeles, over 7000 normal individuals have been typed and available on computer files for matches. Attempts to study non-HL-A specificities for platelets and white cells have been extended and may explain compatible HL-A matches but ineffective transfusions.

White blood cell transfusions can now be tested in preclinical and clinical situations with randomized trials. Preclinically, a dog model is available to study the relative effects of white blood cells and antibiotics as well as various procedures. Clinically, a randomized trial of white blood cells is being conducted to test the efficacy not only by increments in counts but also on infections.

Bone marrow transplantation continues to be an interest of our staff. A preclinical model in dogs has been developed. In dogs, asparaginase has been shown to enhance marrow grafts without added host toxicity. Graft-versus-host disease can be readily produced in dogs and attempts to diminish or modify its devastating effects are being done. Since lymphomas occur in dogs, efforts are underway to see if combined chemotherapy-radiotherapy results can be amplified by allogeneic marrow transplantation. In cyclic neutropenic dogs, marrow transplantation has been curative indicating that the defect can be corrected by replacing stem cells.

Clinically, bone marrow transplantation in the past year was done on seven patients. A total of 19 patients have been transplanted with allogeneic marrows. All patients were refractory to all known chemotherapeutic agents. Despite this, two of the last 10 patients have had very gratifying effects with one long-term 2-year remission. While marrow transplantation in man is not a primary goal of our program,the opportunity and support facilities are suited for a preclinical effort translated into a limited clinical program.

Biostatistical Information Systems Unit

Under Dr. Richard Simon, a very effective statistical support and research unit has been developed. The Unit concerns itself with design, monitoring and evaluation of clinical trials in lung cancer, lymphomas, ovarian, breast, acute leukemia and melanoma. Moreover, the Unit monitors contract supported activities in breast cancer providing program leaders with regular reports. In addition, working with senior staff, statistical research in stratifications and randomization techniques and kinetic models. The kinetic models are being used by others to analyze their data.

Education Program

During the past two years, board certification in Medical Oncology following a defined training program can be obtained. Our fellows and clinical associates have been able to apply their training at the National Cancer Institute to accreditation. This program, composed of a Training and Education Committee, has continued to attract the necessary high caliber fellow to run our clinical program. We feel that the Medical Oncology training experience is an excellent preparation for an academic career in Medical Oncology.

Honors and Awards

Paul P. Carbone, M.D.

1. Invited to serve on the American Board of Internal Medicine's Oncology Test Committee for a period of two years.

2. Appointed Chairman, Nominating Committee, American Society of Clinical Oncology, 1973.

3. Invited to participate in a Cancer Workshop on Non-Hodgkin's Malignant Lymphomas and present a cancer biology lecture, by the Department of Medicine, Washington University School of Medicine, St. Louis, Missouri, July 31 to August 2, 1973.

4. Invited by the Presbyterian Medical Center, Denver, Colorado, to be the Kenneth D. Allen Visiting Professor for 1973, August 4-11, 1973.

5. Invited to speak on "Selection of Treatment Modality in Breast Cancer" at the Three State Regional Meeting of the American College of Physicians, by Dr. T. E. Woodward, Maryland Chapter of FACP, November 3, 1973.

6. Appointed Editor of Medical and Pediatric Oncology: An International Journal, 1974.

7. Reelected Chairman, Eastern Cooperative Oncology Group for a third year.

8. Member of the NIH Medical Board for 1974.

9. Invited to serve on the President's Review Committee for the University of Chicago's Franklin McLean Memorial Research Institute, January 30-31, 1974.

10. Invited to serve on the American Cancer Society's Committee on Diagnosis and Therapy, January 29, 1974.

11. As a member of the Subspecialty Committee on Medical Oncology, invited to meet and discuss the Medical Oncology and Hematology certification for the ABIM, March 8, 1974.

12. Invited to serve on a committee to review research of the Division of Biological Research of the Ontario Cancer Institute, March 20-22, 1974.

13. Invited to participate in the XIX International Bienial Congress of the International College of Surgeons, Lima, Peru, and present two papers on "Breast Cancer: Adjuvant Chemotherapy" and "Intensive Treatment of Patients with Malignant Lymphoma with Combination Chemotherapy", March 24-28, 1974.

14. Scientific Program Co-Chairman, 9th Joint Meeting of the Professional Associations of the U.S. Public Health Service, Washington, D. C., April 8-11, 1974.

15. Invited to speak on "The Combined Modality Therapy of Breast Malignancy" at the Berkshire Medical Center, Pittsfield, Massachusetts, April 20,1974.

16. Invited to present a lecture at St. Elizabeth's Hospital, Boston, Massachusetts, by Dr. F. Stohlman, Director of Medicine and Research, April 22, 1974.

17. Invited to present a lecture to the Southern Society of Clinical Surgeons at NIH by Dr. A. Ketcham, April 23, 1974.

18. Invited to present a lecture on cancer chemotherapy at George Washington University by Dr. H. G. Mandel, April 30, 1974.

19. Invited to participate in the National Blood Club Meeting and speak on "Hodgkin's Disease-Burkitt's", Atlantic City, N. J., May 4, 1974.

20. Invited to present a talk on breast cancer at St. Barnabas Medical Center, Livingston, N. J., May 15, 1974.

21. Invited to present a talk on breast cancer and attend grand rounds at Memorial Sloan-Kettering Institute for Cancer Research, New York, May 23, 1974.

22. Invited to give a lecture for the A.C.P. Course on Cancer Treatment by Dr. John Horton, Albany Medical College of Union University, Albany, New York, June 3-5, 1974.

Publications

1. Holland, J. F., Scharlau, C., Gailani, S., Krant, M. J., Olson, K. B., Horton, J., Shnider, B. I., Lynch, J. J., Owens, A., Carbone, P. P., Colsky, J., Grob, D., Miller, S. P., and Hall, T. C.: Vincristine Treatment of Advanced Cancer: A Cooperative Study of 392 Cases. Cancer Res. 33: 1258-1264, 1973.

2. Costa, G., Engle, R. L., Jr., Schilling, A., Carbone, P. P., Kochwa, S., Nachman, R. L., and Glidewell, O.: Melphalan and Prednisone: An Effective Combination for the Treatment of Multiple Myeloma. Am. J. Med. 54: 598-599, 1973.

3. Rosenblum, A. and Carbone, P. P.: Androgenic Hormones and Human Granulopoiesis In Vitro. Blood 43: 351-356, 1974.

Serial No. NCI-3423
1. Medical Oncology
2. Office of the Associate
   Director
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: In Vitro Marrow Granulocyte Culture

Previous Serial Number: Same

Principal Investigators: Paul P. Carbone, M.D., Joan M. Bull, M.D. and R. J. Mabry, M.D.

Other Investigators: Eleanor Stashick, Wilmarie McKoy, J. Whang Peng,M.D., P. Carmeci, Larry Kun, M.D., and Stephen Rosenoff, M. D.

Cooperating Units: Medicine Branch, MO,DCT,NCI; Pediatric Oncology Branch, MO,DCT,NCI, Cytogenetic Oncology Section, LTCB,ET,DCT, NCI; Department of Technical Development, NHLI, and Department of Radiotherapy, NCI

Man Years:

Total: 5
Professional: 3
Other: 2

Project Description: (1XX)

Objectives:

1. *In Vitro* Marrow Granulocyte Colony Growth in Patients with Breast Cancer.

Marrow function as reflected by peripheral blood values may be abnormal in patients with non-hematologic malignancies with or without bone marrow metastases. *In vitro* granulocyte colony formation (CFU-c) has been shown to be deranged by hematologic malignancies and by antineoplastic chemotherapy. To determine the effect of metastases in bone on CFU-c, aliquots of 4 cc bone marrow aspirates from 22 patients with breast carcinoma were cultured, using a methylcellulose semi-solid system. Thirteen patients with evidence of bone metastases by x-rays, isotope scan, or marrow biopsy were compared with 9 patients free of tumor after surgery and with 14 normal female controls. Patients with metastases had fewer $(2.8 \pm 0.4 \times 10^7)$ cells per cc of marrow aspirate than did normal women $(4.1 \pm 0.4 \times 10^7)$. No differences in colonies per 10^4 marrow cells plated were observed among normals, patients free of tumor and patients with bone metastases. Patients with bone metastases produced significantly fewer colonies per cc of marrow cells plated than did normals (medians, 3500 vs. 9000, $p < 0.05$). No correlation was observed between CFU-c per 10^4 cells of CFU-c per cc and the degree of hematologic toxicity in 12 patients receiving their initial course of combination chemotherapy. CFU-c's from patients with bone involvement by metastatic breast carcinoma grow normally in vitro, but are diminished in concentration within the marrow aspirate.

2. Amplification Assay for Colony Stimulating Activity in Human Serum

Human monocytes are a source of stimulating activity (CSA) for in vitro growth of granulocyte-monocyte colonies. To study how normal serum factors modify feeder layer (CSA) we prepared monocyte-rich feeder layers from one normal human bone marrow and five peripheral blood samples and added to the agar layer either human serum or, as control, fetal calf serum, before overlaying mouse bone marrow cells in methyl cellulose. At 4-6 x 10^5 cells per plate feeder cells stimulated only 0-50 mouse colonies per 10^5 mouse cells plated. Human serum in agar produced only 0-10 colonies per 10^5 cells per 0.1 cc serum. In all experiments, addition of serum to the monocyte-rich feeder layer resulted in more colonies (range 30-128 colonies per 10^5 cells) than expected from simple addition of effects. Incubation of feeder layers for 7 days, followed by freeze-thawing, and then addition of serum showed similar amplification of colonies. Supernatant of culture medium incubated with 5 x 10^5 monocytes per cc for 7 days produced 40-60 colonies per 10^5 cells in excess of addition when later mixed with 0.1 cc serum in agar underlayer. Dilute conditioned medium from various human sources (embryo kidney cells, blood leukocytes, bone marrow) in the presence of 0.1 cc test normal serum produced a 4- to 10-fold amplification of human serum stimulating activity. The amplification was abolished at high concentrations of added conditioned media. We interpret the evidence to indicate that amplification of low levels of activity present in normal serum results from combination of subthreshold activity present in both serum and feeder layers or conditioned medium. This may provide the basis of a sensitive assay for stimulating activity in normal serum.

3. Cytogenetics and In Vitro Marrow Pattern

Patients with preleukemia as well as acute leukemia are known to produce abnormal marrow colony formation. The abnormalities follow 3 patterns. Those patterns are a) absence of normal colonies, b) cluster formation only, or c) a superabundance of colonies. Lymphoma patients treated with both combination chemotherapy and extensive radiotherapy have developed abnormal marrow cytogenetics. A certain number of patients treated with whole body radiation and chemotherapy in the past are being prophylactically examined with marrow cytogenetic analysis by Dr. J. Whang Peng, marrow histology and, in addition, in vitro marrow culture. To date a single patient developed cluster formation at a time when his cytogenetic pattern was noted to be quite abnormal. The study is ongoing.

4. *In Vitro* Granulocyte Colony Production (CFU-c) and Direct Cytogenetic Studies of Patients with Chronic Myelogenous Leukemia (CML) Treated to a Philadelphia Chromosome Negative (Ph^1-)Status.

The methylcellulose *in vitro* granulocyte culture system was used to examine the colony production and cytogenetic status of the bone marrow and the peripheral blood of 3 patients with Ph^1+CML in long-term Ph- remission. These patients had been treated early in their disease onset with a combined modality regimen consisting of splenic radiotherapy, splenectomy, and an intense 4-drug chemotherapy course. The 3 patients had been in long-term remission on hydroxyurea maintenance for 6-24 months prior to the culture studies. The results of the analyses are:

Patient	Direct Cytogen.	Colony Growth (Normal 1.8-6.4/10^4 NCP)	Marrow Cytogen.	PB Growth (15-100/10^5 NCP)	PB Cytogen.
MT	32 Ph+/38 Total Metas.	Clusters only	27 Ph^1+/27 Total Metas.	0	-
AR	0 Ph^1+/36 "		0 Ph^1+/22 Total Metas.	0	-
RR	1 Ph^1+/63 "	Normal	3 Ph^1+/36	Normal	0 Ph^1+/45 Total Metas.

The cytogenetic analysis of cultured marrow confirmed and extended the data obtained by direct marrow cytogenetics. It is of interest that the circulating CFU-c cell in the peripheral blood of patient RR is Ph^1- whereas the patients' marrow granulocyte colonies are both Ph^1+ and Ph^1-.

5. The Effect of Androgenic Steroids on Human Granulopoiesis *In Vitro*.

Androgens play a major role in the therapy of metastatic breast carcinoma and aplastic anemia. It is well documented that the non-synthetic androgen, testosterone propionate increases erythropoiesis, however, the androgen effect on granulocyte proliferation is less well documented. The semi-solid *in vitro* granulocyte culture technique allows the direct assay of the effect of androgens on human marrow leukopoiesis. Using non-fractionated marrow aspirates from 6 normal individuals and 2 patients with breast carcinoma, it was found that the addition of testosterone propionate (10^{-8} - 10^{-11} molar) increased the granulocyte colony formation (CFU-c) to mean 29% (range 18-54%) above the control values. The addition of 7β, 17 α dimethyl testosterone (Calusterone) a new anabolic weakly androgenic steroid increased the CFU-c to mean 36% (range 22-61%). And Δ-1-testololactone a non-masculinizing androgen derivative effective in the treatment of breast carcinoma also increased the CFU-c to mean 26% (range 16-48%). The addition of an estrogen (diethylstilbesterol) (10^{-6}-10^{-10} molar) did not significantly effect colony production. The androgenic agents increased the CFU-c of whole, non-fractionated marrow and did not stimulate growth of the non-adherent marrow fraction in the absence of the adherent marrow cells that endogenously produce colony stimulating factor (CSF).

These data suggest that 2 synthetic androgenic steroids are as leukopoietic as testosterone propionate and that the androgenic steroids do not act by stimulating the CFU-c cell directly and may stimulate the proliferating granulocyte cell by affecting the marrow cells that produce CSF.

6. Testing of CSF.

Abbott Laboratories, under contract with the NCI, is establishing the production of large quantities of human granulocyte colony stimulating substance (CSF) for possible clinical application. This laboratory is involved in testing the activity of these preparations in cooperation with investigators from St. Elizabeth's Hospital, Boston, Massachusetts and the Toronto Cancer Institute, Toronto, Canada.

7. The Peripheral Blood Colony-Forming Cell.

When a ficoll-hypaque sedimentation gradient is made of peripheral blood, granulocyte colony formation is found with *in vitro* culture (circ.CFU-c). The circulating colony forming cells represent a composite sample of the entire bone marrow's production of granulocyte colonies. These circulating cells are being examined in patients with solid tumors and lymphomas in the presence and absence of marrow invasion with tumor and with the comparative stresses of limited field, whole body radiation, and with chemotherapy. Preliminary results indicate that in patients with no prior therapy there is a 3-5-fold increase in the number of circulating CFU-c with limited field radiotherapy, whereas in those patients with extensive prior therapy the circulating CFU-c falls to zero with even limited radiotherapy indicating a poor marrow reserve.

8. The Effect of Chemotherapy on the Kinetics and Proliferative Capacity of Normal and Tumorous Tissues *In Vivo*.

The proliferative state of a given tissue is a major determinant of its sensitivity to both phase specific and cycle specific chemotherapeutic agents. To study the extent of injury induced by antitumor agents to normal and tumorous tissues, a technique for following DNA synthesis as reflected in the incorporation of tritiated thymidine (^{3}H-TdR) into DNA was compared to the conventional autoradiographic technique of the labelling index (LI) and to the functional kinetic technique of granulocyte colony formation *in vitro*. Alterations in DNA synthesis induced by a single dose of cyclophosphamide in normal and tumorous tissues *in vivo* paralleled in many respects the changes seen when the more time consuming techniques of the LI or granulocyte colony formation were employed. However, the recovery of granulocyte colony formation after cyclophosphamide therapy lagged behind the recovery of DNA synthesis in the bone marrow, obscuring a kinetic event of potential therapeutic significance. The determination of DNA synthesis simultaneously in normal and tumorous tissues *in vivo* was easy to perform and supplied therapeutically pertinent results comparatively quickly.

Future Course of Project:

1. Agents modifying cell cycle characteristics of bone marrow stem cells *in vitro*. Cyclic AMP has been shown to promote the entrance of CFU-s with S phase of cell cycle. Work is underway to establish whether cyclic nucleotides have an analogous role in regulation of CFU-c varieties. The effect of cyAMP, cyGMP, imidazole, insulin and theophyllins on the fraction of CFU-c varied by exposure to hydroxyurea or to tritiated thymidine is being assessed.

2. Continued work with the circulating granulocyte colony cell in disease states, and modified by therapy is ongoing.

Honors and Awards:

Dr. Joan M. Bull

1. Studies in granulocyte precursor cell growth in patients with acute leukemia (lecture), presented at Symposium of Prognostic factors in acute leukemia, held at Schloss Reisensburg, Reisensburg, Germany, Oct. 1973.

2. Sex differences in normal human granulopoiesis *in vitro*, presented at the meetings of the American Society of Hematology, Chicago, Dec. 1973.

3. Altered *in vitro* granulopoiesis in the presence of leukemic and normal marrow cells, presented to the Washington Blood Club, Dec. 1973.

4. The effect of exogenous steroids on human granulopoiesis *in vitro*, presented at the EORTC Stem Cell Club, Manchester, England, March 1974.

5. Presented paper at the meetings of the American Association for Cancer Research, Houston, Texas, March 1974.

6. Invited to give a talk at Brookhaven National Laboratories on The Effect of Steroids on *In Vitro* Granulopoiesis" Jan. 1974.

Dr. R. J. Mabry

1. *In vivo* marrow granulocyte colony growth in patients with breast cancer. Presented at the meetings of the American Association for Cancer Research, Houston, Texas, March 1974.

Publications:

1. Moore, M. R., Jones, S. E., Bull, J. M., Williams, L. A., and Rosenberg, S. A.: MOPP chemotherapy for advanced Hodgkin's disease. Cancer 32: 52-60, 1973.

2. Bull, J. M., Duttera, M. J., Northup, J. D., Henderson, E. S., Stashick, E. D., and Carbone, P. P.: Serial *in vitro* bone marrow culture in acute myelocytic leukemia. Blood 42: 679-686, 1973.

3. Duttera, M. J., Bull, M., Stashick, E. D., and Carbone, P. P.: Serial *in vitro* bone marrow culture in acute lymphocytic leukemia. Blood 42: 687-699, 1973.

4. Bull, J., Rosenblum, A., and Carbone, P. P.: In Vitro Culture Studies in Patients with Acute Myelocytic Leukemia, *in* Proceedings of the Second International Workshop, pp. 335-340. DHEW Publication No. 74-205, December 1973.

Serial Number NCI 3024
1. NCI-VA Medical Oncology Branch
2. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1973 through June 30, 1074

Project Title: Chemotherapy of Cancer

Previous Serial Number: 3024

Principal Investigator: Oleg S. Selawry

Other Investigators: Martin H. Cohen, Patrick J. Creaven, Mary J. Matthews, Larry Allen, Lawrence E. Broder, Byron E. Fossieck, Suryabala Kanhouwa, Aron Primack, Marc Straus, Francisco Tejada, Paul Hamosh

Cooperating Units: The NCI-VA Medical Oncology Branch represents a joint effort of the National Cancer Institute and the Veterans Administration. Funds are provided by the National Cancer Institute, administered by the Veterans Administration Central Office. Facilities and basic care are provided by the Veterans Administration Hospital in Washington, D.C.

Man Years:

Total:	78 7/10
Professional:	9 7/10
Other:	69

Project Description:

Objectives:

A. Research

1. Introduction of new anticancer agents into clinical use and pharmacology of antitumor agents.

2. Improved use of chemotherapeutic agents of established value.

3. Improvement of treatment for patients with bronchogenic carcinoma.

B. Service

1. Provide medical oncology consults to patients at the Washington Veterans Administration Hospital.

2. Provide postgraduate training for the subspecialty of medical oncology.

3. Particiapte in teaching activities of the Medical Service, Washington Veterans Administration Hospital, and of the Department of Medicine at George Washington University.

Summary of Major Accomplishments

The Medical Oncology Branch expanded its efforts in the areas of clinical pharmacology and in multidisciplinary treatment of solid tumors, with emphasis on bronchogenic carcinoma:

Eight of 10 clinical pharmacology studies including 198 patients were finished, two are close to conclusion. Four studies included new drugs: 1) ICRF 159, recommended dose 1 gm/m^{2}t.i.d. p.o. once weekly (Dr. Creaven and staff); 2) isofosfamide, recommended dose 5 gm/m^2 i.v. q 3 weeks (Dr. Cohen) (acetylcysteine does not affect alkylating activity--metabolism differs for cyclophosphamide (Drs. Creaven, Allen)); 3) epipodophyllotoxin-ethylidene glucoside (VP-16), recommended dose 290 mg/m^2 i.v. once weekly (Dr. Creaven,staff); 4) thalicarpine, maximum tolerated dose 1.4 gm/m^2 for single i.v. infusion (Dr. Creaven, staff). These studies included determination of pharmacologic parameters, drug metabolism, and, for thalicarpine, mode of action (Drs. Creaven, Allen, staff) and were facilitated by computer assisted pharmacokinetic modeling (Dr. Allen). Two studies included combinations of drugs: 1) CCNU + caffeine + chlorpromazine (Dr. Cohen), no change in toxicity of CCNU, study currently advanced by ECOG; 2) gleomycin, 30 mg/m^2/week i.v. X 6 + streptozotocin, 1 gm/m^2/week i.v. X 6 (Drs. Hansen, Selawry): both "non-myelosuppressive" drugs can be combined at full dose, with reversible toxicity. One study includes tritiated thymidine, as baseline for cell kinetic studies (Dr. Straus). Three studies focused on pharmacology of established antineoplastic agents: 1) epipodophyllotoxin-thenylidene-glucoside (VM 26) is under investigation because of the marked quantitative difference in clinical tolerance when compared to VP-16 (v.s.) (Drs. Creaven, Allen); 2) intestinal absorption of methotrexate was found to be reduced to 70-80% of pretreatment values in 3 of 7 patients on oral antibiotics while in reverse isolation (Drs. Cohen, Creaven), 3) biliary excretion of methotrexate was found to be minimal (Dr. Creaven).

Superior survival and superior tumor regression of patients with bronchogenic small cell carcinoma and adenocarcinoma were confirmed (Drs. Hansen, Selawry, staff) for a sequence of two 3-drug combinations as compared to the same six drugs used in three 2-drug combinations. Responders lived substantially longer than nonresponders.

This study served as baseline for nine new successor studies, adjusted to cell type and stage of lung cancer. In one of these studies it has been noted that patients in reverse isolation (Laminar Air Flow room), and on oral antibiotics, tolerate twice as much parenterally administered drug than patients on the open ward (Drs. Cohen, Fossieck). More patients will be needed for judgment of therapeutic effects. Forty-eight new patients have been included in these studies.

Group studies of ALGB (Dr. Tejada) and of ECOG (Dr. Cohen) were supported with 10 patients, The Working Party for Therapy of Lung Cancer, 28 patients.

Ancillary studies were directed toward surveillance of infectious complications (Dr. Fossieck), diet, and calorie input (Dr. Cohen, Ms. Arkwright, staff) and social problems (Ms. Miller).

The expertise and dedication of the nursing staff (Ms. Boyer, Ms. Tvardzik, staff) formed the very basis of our therapeutic and investigational efforts for hospitalized and ambulatory patients, including

400	admissions
432	discharges
1738	clinic visits
280	consults
1425	ward and OPD procedures (bone marrow examinations, etc.)
2525	special blood specimen collections

Quantitation of tumor regression and progression is advanced by serial endobronchial (Drs. Cohen, Kane), cytologic (Drs. Matthews, Kanhouwa), and marker substance (Dr. Broder) follow-up. Computerized image processing of chest films failed to show improved quantitation of changes in tumor size but led, instead, to the exploration of "ACTA," automated, computerized, transverse axis tomographic scanning, with promising initial data (Dr. Hamosh).

Conceptual aspects of cancer therapy focus on cell kinetics (Drs. Straus, Tejada, Kane, staff), cell cultures and marker substances (Dr. Broder, staff), cell membrane active agents as adjuvants to chemotherapy (Dr. Cohen), and drug distribution in tumors (Dr. Creaven, staff), with important achievements as outlined in the individual reports. It is expected that the interplay between pharmacokinetics, cell kinetics, and marker substances, as studied _in vitro_, will be advanced exploration of their interplay _in vivo_.

The Pathology Section (Drs. Matthews, Kanhouwa) extended its activities toward clinic and morphology. Major studies focused on actual extent of disease as opposed to clinically recognized extent of lung cancer in patients who died within two months after surgery (in cooperation with Dr. Higgins). A survey of 418 autopsies of lung cancer patients elucidated the patterns of spread by cell type. Sputum cytology was found to permit reliable diagnosis by cell type in patients with all but poorly differentiated epidermoid carcinoma, adenocarcinoma, and large cell carcinoma.

Last, but not least, the program was strongly supported by the spirited dedication and expert work of our clinical associates, oncology fellows, residents, interns, and students. Specifically, our trainees developed and initiated three Phase II studies in lung cancer, and presented an admirable series of seminars on major cancers by organ site. The training program, with clinical and preclinical presentations, was under the leadership of Drs. Cohen and Straus, with full support by the entire medical staff.

The scientific and educational efforts of the Branch are reflected in 45 publications, 12 abstracts, and 37 extramural and 21 intramural lectures and seminars.

Detailed Reports

Stage II, 2AX-Ic LA:

A Kinetic Analysis of Sarcoma-180 Tumor Growth

(Marc Straus and Robert Kane)

A comprehensive *in vivo* and *in vitro* model has been developed to characterize kinetics of tumor growth. S-180 tumor is grown in CDF mice in ascites and solid tumor form, and in soft agar *in vitro*. (The latter is a collaborative effort by Dr. S. Shackney.) The ascites tumor has given reproducible growth characteristics. After tumor inoculation and *in vivo* labelling with ^{3}HTdR, daily ascites cell counts and DNA content distributions, early and late PLM and continuous infusion curves, and specific activity of DNA were determined. PLM curves were evaluated at multiple thresholds and exposure times to facilitate cell cycle analysis. The cell cycle analysis shows characteristic changes in generation time and cycle phases with tumor growth. S-phase specific agents are presently being added and their effects on tumor growth and cell cycle parameters as a function of time and dosage are being quantitatively measured. These data will be compared to data from the human *in vivo* kinetics studies.

An Analysis of Solid Tumor Growth in C57Bl Mice

(Marc Straus and Stanley Shackney)

A mathematical analysis of solid tumor growth was made, based on data previously obtained. CA755 trocar implants were placed S.C. in the abdominal area of C59Bl mice. Each day thereafter, 20-30 mice were sacrificed, the tumor removed and weighed. In a number of mice, daily caliper measurements of the tumor were made and volume was calculated. The pattern of tumor growth obtained by surgical removal was sigmoidal. The smallest measurable tumors were 25 mg. Caliper measurements were only possible above 1 gm volumes and resulted in higher volume determinations. Gompertzian growth which is assumed to occur from caliper data is misleading when extrapolated to early growth. Reasons for differences in measurements by various methods were analyzed.

S-180 Tumor in vitro Assays

(Marc Straus and Stanley Shackney)

A soft agar system was developed to test plating efficiency of S-180 tumor cells. The effects of various concentrations of hydroxyurea and Ara-C for varying lengths of time on the plating efficiency were measured. These data will be analyzed together with S-180 tumor cell kinetics in in vivo systems.

Stage II, 2AX-II LA:

Enhancement of the Antitumor Effect of Nitrosoureas by Caffeine and Other Agents

(Martin Cohen and Susan Krezoski)

If mice bearing L1210 leukemia are treated 7 days after inoculation of 10^5 L1210 cells by BCNU in optimal dose (30 mg/kg) or with BCNU and caffeine, the median survival is 8 days, the same as that of untreated controls. If hydroxyurea is started on day 7, 4 hours before BCNU and repeated 10 minutes before BCNU. the median survival is increased to 25 days and there are 30% cures in contrast to 1 to 3% cures with BCNU alone. Other hydroxyurea schedules are less effective. Hydroxyurea alone produces a 1-day increase in median survival.

Stage II, 2B2-I-a LA:

Optimal Conditions for Combination Chemotherapy in Spin Culture Systems

(Martin Cohen, Lawrence Broder and Cleo Thornton)

Experiments using L1210 cells in culture and the Himmelfarb-Thayer spin filter apparatus are designed to demonstrate optimal timing for combination chemotherapy in culture. Nutrients to cultures are varied to develop a "fast" and "slow" growing cell line. Cell parameters, including DNA, RNA and protein synthesis are studied for each drug treatment and optimal time for the second drug in the combination are determined from the changes in these results from the initial treatment. Combination of hydroxyurea and nitrosourea in cell culture are being analyzed. Currently the development of the Himmelfarb -Thayer apparatus is being worked on and the implementation of a human cell line for spin culture experiments is being considered.

Stage IV, 4X1-b LA:

Phase I Clinical Trial of 4' Demethylepipodophyllotoxin Ethylidene Glucoside (VP 16-213, NSC 141540)

(Patrick Creaven and medical staff)

Forty patients were entered into a Phase I clinical trial of VP 16-213 at a starting dose of 20 mg/m^2 per week by intravenous infusions in 250 ml m of 5% dextrose given over 30 min. every week for 6 weeks. The dose was escalated by a modified Fibonacci search in 8 dose steps to a final dose of 290 mg/m^2 per week with downward dose modification according to extent of toxicity. Easily controlled nausea and vomiting occurred at doses from 20 to 130 mg/m^2 per week. Mild leukopenia also occurred in some patients. Consistent leukopenia was seen at doses 170, 220, and 290 mg/m^2 per week. The median nadir of WBC was 3150/cu.mm at 170 mg/m^2; 3050/cu.mm at 220 mg/m^2; and 2770/cu.mm at 290 mg/m^2. Toxicity occurred at a median of 2 weeks at doses of 170 and 220 mg/m^2 and 1.5 weeks at a dose of 290 mg/m^2. Thrombocytopenia was seen in 0 of 7 patients at 170 mg/m^2, 1 of 7 patients at 220 mg/m^2, and 2 of 9 patients at 290 mg/m^2. The recommended dose for Phase II clinical trials is 290 mg/m^2 per week with downward dose modification according to the degree of leukopenia. Begun in March, 1972, the study was concluded in January, 1974.

Phase I Clinical Trial of Isofosfamide

(Martin Cohen and medical staff)

Thirty-five patients were admitted to the study. The dose level started at 200 mg/m^2, escalated to 5000 mg/m^2. Dose limiting side effects were hematuria and hemorrhagic cystitis in 80% of patients receiving doses higher than 3800 mg/m^2. Complications were not seen in patients who maintained normal renal flow. Cystitis could be prevented by bladder irrigation.

Phase I Clinical Trial of 1,2 Bis-(3,4 Dioxopiperazin-1-yl) Propane (NSC 129,943; ICRF 159)

(Patrick Creaven and medical staff)

Phase I evaluation of single dose and two weekly schedules of ICRF 159 was carried out in 38 patients. Initial dose was 1.0 g/m^2, escalated to a final dose of 10.5 g/m^2. All doses above 1.0 g/m^2 gave mild hematologic toxicity, not dose related and inconsistent, occurring in only 3 of 9 patients receiving the highest dose. Schedule I: 6 patients received 3.0 g/m^2 as a single dose weekly for 6 weeks. A median nadir of WBC of 3800 was observed at a median of 3 weeks. Thrombocytopenia was not seen. Schedule II: 10 patients received 3.0 g/m^2 once weekly for 6 weeks, each dose divided into three equal parts given at 6-hour intervals (9 A.M., 3 P.M., 9 P.M.). Leukopenia, occurring in 9 of 10 patients, showed a median nadir of 2100/cu.mm. Thrombocytopenia occurred in 3 of 10 patients. The recommended dose and schedule for Phase II studies: 3.0 g/m^2 per week in subdivided doses, as in Schedule II. Since it was concluded that failure of single doses to give consistent toxicity might be related to poor absorption of the drug, this was investigated (see: Clinical Pharmacology of ICRF 159, Stage IV, 4X2-I). The study was concluded.

Phase I Clinical Trial of Thalicarpine (NSC 68075)
(Patrick Creaven and medical staff)

Initial dose was 200 mg/m^2 in 500 mm of 5% dextrose in water given by infusion over 1 hour, escalated to a dose of 1070 mg/m^2. A number of patients complained of pain in the arm as doses of 1070 mg/m^2 and above were given by a 2 hour infusion in one lather of 5% dextrose in water. All patients at doses above 1070 mg/m^2 complained of some degree of pain or irritation in the arm of infusion and developed mild to moderate drowsiness during the latter part of the infusion, lasting for 2 to 24 hours. At a dose of 1900 mg/m^2, 1 patient became markedly unresponsive for a period of 24 hours. Following this, there was a considerable increase in psychotic behavior over that which had existed before treatment. The patient subsequently developed seizures. His EKG showed nonspecific STT wave changes and low voltage. The other patient also developed nonspecific STT wave changes and low voltage and considerable weakness in the three days following treatment. It was concluded that a dose of 1900 mg/m^2 probably represented a toxic dose high in humans. The safety of administering 1400 mg/m^2 is currently being investigated. Five patients have received this dose; four are evaluable. There is evidence of toxicity. Twenty-nine patients have been entered on the study; no deaths were directly attributable to drug administration. The study will continue with an investigation of weekly administration.

Combination of CCNU (NSC 79037) + Caffeine + Chlorpromazine
(Martin Cohen and medical staff)

Twelve patients have been entered on protocol in which chlorpromazine, 50 mg/m^2, was given 6 hours before CCNU, 30 mg/m^2 p.o., followed by caffeine, 600 mg/m^2 S.C. This treatment was repeated every 4 weeks. There has been no increased hematologic toxicity over that expected with CCNU alone. There was 1 partial response and 2 patients with static disease for 3 and 5 months in three patients with melanoma and 1 response of 2 patients with brain tumor. Treatment was well tolerated. With chlorpromazine, only 1 patient developed postural hypotension, reversible on lying down. There was no toxicity attributable to caffeine.

Phase I Study of Bleomycin and Streptozotocin in Patients with Bronchogenic Cancer

(Oleg Selawry)

When both drugs are given at full dosage there is consistent, reversible toxicity. Toxic manifestations included vomiting (8), transient increase of creatinine (1.8, 2.1, 2.8 mg%, respectively, in 3 patients), dermatologic toxicity (3), fever (2), and thrombocytopenia (2). Two patients with epidermoid carcinoma showed transient, objective regression of their tumor lesions, including 1 of 3 patients with esophageal cancer and 1 of 1 patient with cancer of the floor of the mouth. Non-responders included 2 patients with epidermoid cancer of the lung, 3 patients with large cell carcinoma of the lung, one hepatoma and one carcinoid. The study was finished.

The Uptake, Metabolism, and Excretion of Tritiated Thymidine in Man

(Marc Straus, Francisco Tejada, Susan Krezoski and Lewis Battiste)

Patients were injected with .2 mCi/kg ^{3}HTdR in order to obtain adequate radioautographs. A metabolic study was developed to assess the potential radiotoxicity of these doses of ^{3}HTdR to the patients and to investigators in close proximity to the patients. Serial samples of plasma, urine, sputum, and air vapor were measured for tritium activity in 12 patients over 2 to 12 week periods. Fifty-five percent of the total injected activity was excreted in the urine within 10 days. Within 10 minutes plasma activity was 5-10% of the injected level. After 1 to 2 days, activity in all compartments were equal and decayed at a $T_{1/2}$ of 10-12 days, the biological half-life of tritiated water. Approximately 10% of activity was not excreted and was potentially incorporated into DNA. The radiation hazard from this dose is $<$5 rads. There is no radiohazard to investigators when simple precautions are followed.

Stage IV, 4X2-I LA:

Clinical Pharmacology of VP 16-213

(Patrick Creaven, Larry Allen and Dorothy Hoyt)

Twenty-two patients have been studied at doses 70 to 290 mg/m^2 using tritium labelled VP 16-213 (4' demethylepipopophyllotoxin-9-(4,6-0-2 ethylidene- -D-glucopyranoside). Total recovery of urinary radioactivity was 52.2 ± 16.1%. Urinary radioactivity was 60% unchanged VP 16, determined chromatographically. Only minor amounts of the drug were found in the feces. Plasma levels of unchanged drug determined at 0-48 h post infusion fitted in most cases a biexponential decay curve (by non-linear least squares regression on an IBM 360 computer). The half-life of the initial and terminal phases were 2.34 ± 0.69 h and 11.43 ± 2.95 h. At 24 h post infusion plasma radioactivity was 54 to 78% of unchanged drug. The levels of drug in the CSF were extremely low (0157% plasma levels at 2.6 h after dosage and 7.3% of plasma levels at 12 to 54 h post infusion). The highest level of the drug measured in the CSF was 0.13 μg/ml. The project is continuing with an attempt to account for the radioactivity not recovered in the urine and with studies of protein binding of the drug.

Clinical Pharmacology of ICRF 159
(Patrick Creaven, Larry Allen and Dorothy Hoyt)

The absorption of ICRF 159 labelled with $[^{14}C]$ in the carbon groups was studied in 6 patients who received the drug at a dose of 10.5 g/m^2. Mean absorption as measured by total $[^{14}C]$ recovered in the urine was 8.5 ± 3.0%. Four of these patients were subsequently restudied at a dose of 3.0 g/m^2 and mean recovery of radioactivity in the urine was 22.7 ± 10.5% of the administered radioactivity. Seven patients were subsequently studied at a dose of 3.0 g/m^2 given in divided doses of 1.0 g/m^2 times 3 at intervals of 6 hours. Total urinary recovery of radioactivity was 51.7 ± 8.9%. The area under the plasma concentration curve for radioactivity was essentially the same in patients receiving 10.5 g/m^2 and the same patients restudied at 3.0 g/m^2. The time from dosage to peak plasma concentration did not differ at the two dose levels. Although measurement of total recovery of urinary radioactivity is not an ideal method of measuring bio-availability, all the evidence seems to point in the direction of a limitation of absorption of the drug at dose levels above about 1.0 g/m^2, paralleling the clinical experience with toxicity of large single doses. The limited absorption of this drug has since been confirmed elsewhere in animal studies. The project is continuing with a study of plasma decay of unchanged ICRF 159 and its metabolites and the isolation and identification of the urinary metabolites of the drug.

Pharmakinetics Biopharmaceutics Mathematical Modelling of New Anticancer Agents in Man
(Larry Allen and Patrick Creaven)

Pharmakinetics development of mathematical models adequately describes data collected from humans for the absorption, distribution, and elimination of new anticancer agents in man from data collected by using radioactive tracers, gastography, or other analogical methods. Samples from blood, urine, and other accessible compartments are used to construct the mathematical concept for the distribution of the drug and its elimination. A pharmakinetic model for ifosfamide in man have been developed and is submitted as an abstract to the International Cancer Congress in Florence, Italy, and to a journal in the cancer field. Use is made of the deck system 10 and IBM 370 computers for evaluation, estimation of data, and construction of the model. Statistical techniques are used to evaluate the model for its variability and determination of the appropriate model.

Methotrexate Absorption in Patients on Oral Prophylactic Antibiotics

(Martin Cohen and Patrick Creaven)

Patients receiving oral antibiotics as part of the Laminar Air Flow Reverse Isolation Protocol were studied from the standpoint of absorption of oral radiolabelled methotrexate before and during the period of time on gut sterilization. Absorption prior to the start of antibiotics was uniform in the 7 patients studied. On antibiotics absorption became variable with 3 of the 7 patients having a 20 to 30% decrease in absorption while other patients maintained normal absorption.

Clinical Pharmacology of Isophosphamide

(Patrick Creaven, Larry Allen and Dorothy Hoyt)

Alkylating activity during nine 8-hour periods following infusion of isophosphamide at doses from 2900 to 5000 mg/m^2 was studied in 12 patients. In 5 patients receiving cyclophosphamide, 1100 mg/m^2, it was found that total urinary alkylating activity for 1100 mg/m^2 of cyclophosphamide was greater than that for 3800 mg/m^2 of isophosphamide, but was less than that for 5000 mg/m^2 of isophosphamide. The concentration of urinary activity was higher in patients who developed hemorrhagic cystitis. Addition of acetylcysteine to the bladder washout did not alter the total alkylating activity. Plasma alkylating activity was studied in patients receiving 3800 and 5000 mg/m^2 isophosphamide and those receiving 1100 mg/m^2 cyclophosphamide. Plasma alkylating activity persisted for longer periods and was at higher levels after 5000 mg/m^2 of isophosphamide than after 1100 mg/m^2 of cyclophosphamide. Using isophosphamide randomly labelled in the chloroethyl side chanins with [^{14}C], the half-life of isophosphamide was found to be 13.79 hours, a value much longer than that reported for cyclophosphamide; 61% of the total urinary radioactivity was accounted for by unchanged isophosphamide. In studies with cyclophosphamide by others, the proportion of cyclophosphamide excreted unchanged was found to be only 10-20%. These results are compatible with our previous findings of a lower affinity of isophosphamide than of cyclophosphamide for the microsomal drug metabolising enzyme system. The finding of measurable amounts of isophosphamide in the cerebrospinal fluid can be correlated with the observation of infusion in some patients receiving high doses of this drug. However, no alkylating activity was found in the cerebrospinal fluid, indicating minimal penetration into the central nervous system. The study is concluded.

(Allen and Creaven, 1972, 1972, 1972, 1973, 1973; Allen, et al, 1973, 1973)

Clinical Pharmacology of Thalicarpine

(Patrick Creaven, Larry Allen and Carole Williams)

Fourteen patients have been studied with tritium labelled thalicarpine at doses from 300 to 1900 mg/m^2. Eight to 20% of total administered radioactivity was recovered in the urine up to 69 hours post dosage. The fate of the blot of the radioactivity administered is still undetermined. The administered radioactivity is excreted largely as unchanged thalicarpine in the 0-5 hour period post infusion, at later times proportion of metabolised thalicarpine rises, and at 70 hours only 10-30% of the urinary radioactivity is unchanged thalicarpine. Plasma half life of the decay of radioactivity is long, being 2 to 5 days for the beta phase of the decay. Penetration into the cerebrospinal fluid is considerable, being up to 20% of plasma levels of radioactivity at 24 hours post infusion. The chemical identity of the material penetrating the CSF is so far undetermined. The project is continuing.

Clinical Pharmacology of VM-26

(Patrick Creaven, Larry Allen and Dorothy Hoyt)

Five patients have been so far studied using tritium labelled VM-26 (4' demethyl-epipodophyllotoxin-9-4,6-0-2-thenylidene- β-D-glucopyranoside). Total recovery of radioactivity in the urine is 35-40%. The fate of the rest of the radioactivity is so far undetermined. After 12 h post infusion, all the urinary radioactivity is represented by metabolite; there being essentially no unchanged drug in the urine at this time. Measurable levels of radioactivity in the cerebrospinal fluid have been found in about only 1 of 3 patients studied so far; this patient had had extensive brain surgery. The results and data with this drug are preliminary at this time. The study continues. A major purpose of the study is to determine possible pharmacological and pharmacokinetic reasons for the large difference in moderately toxic dose in humans with VM-26 (67 mg/m^2/week) and VP 16-213 (290 mg/m^2/week). To this end, plasma protein binding, urinary excretion, plasma decay, and metabolism of the 2 drugs are being studied in parallel.

Stage IV, 4X2-II LA:

Data Processing: Clinical and Laboratory Experiments in Oncologic Pharmacology

(Larry Allen, Patrick Creaven and Delores Ellison)

The conversational programming system of the IBM 370/65 is used for general laboratory analysis, including data conversion in radioisotope assays, kinetics and statistics of simple enzyme reactions, estimations of protein binding constants, and for programs written to simulate multiple dose versus time profiles. Additional programs in CPS-basic are used for T-test, chi square, and linear plotting. The Wylbur system is used to edit, modify and/or enter data for pharmacokinetic analysis, to punch cards, off-line and access the pre-packaged statistical routines. The DEC System-10 computer, on-line with the Textronics-4010 terminal, is used for developing multi-compartment pharmacokinetic models, mathematical analysis of drug protein binding complexes, and advance analysis of enzyme kinetics. The timesharing environment of the DEC-10 has been found ideal and cost saving for advanced scientific applications in pharmacology.

Stage IV, 4X2-III LA:

Antineoplastic Drug Protein Interactions
(Larry Allen, Patrick Creaven, Dorothy Hoyt, Carole Williams and Carole Marcks)

Systematic analysis of new anticancer drug protein binding interactions are being investigated as to their molecular binding properties and quantitations of these properties through computer modelling by either structured or stepwise equilibrium analysis. When possible, structural analogs of these antineoplastic agents are compared for details through molecular aspects of protein binding, not only to plasma protein but to other binding components, for their influence on absorption and distribution in bile elimination restrictions to effective anticancer therapy.

Inhibition of Glucuronyltransferase by Thalicarpine (NSC 6807)
(Larry Allen, Patrick Creaven and Carole Williams)

The enzyme kinetics of phenolphthalein and p-nitrophenol glucuronic acid conjugation inhibited by thalicarpine and four structurally related bis-benzyl isoquioline derivatives using rabbit liver glucuronyltransferase was studied. The inhibition seemed to be confined primarily to the substrate binding site for phenolphthalein and only secondarily to the coenzyme binding site for UDP-glucuronic acid. Thalicarpine inhibition was greater for phenolphthalein than for p-nitrophenol conjugation. With one exception, the order of increasing inhibition of phenophthalein conjugation was in the same order as increasing stereochemical restriction about the benzyliso-quinoline nitrogen for four derivatives. The project has been completed.

Molecular Pharmacology of Thalicarpine (NSC 68075)
(Larry Allen, Patrick Creaven, Cary Owens and Carole Marcks)

The plant alkaloid thalicarpine inhibits the synthesis DNA, RNA, and protein in cultured L1210 cells. Significant inhibition of thymidine incorporation into L1210 DNA and uridine into RNA by 100μM thalicarpine occurs within 20 minutes. Whereas inhibition of DNA synthesis is only partially reversible, that of RNA is nearly reversed when the cells are washed free of the drug. Thalicarpine also inhibits early stages of nucleotide triphosphate biosynthesis. Thalicarpine is notable for its multiple and diverse modes of action, all of which may be responsible for its chemotherapeutic effect. This study has been continued by investigating the effect of thalicarpine on isolated DNA polymerases from yeast, E. coli and calf thymus. In addition, studies are in progress on the individual quantitation of nucleotide precursors influence by thalicarpine in L1210 mouse leukemia cells grown in suspension culture. The study continues.

Interaction of Thalicarpine with the Microsomal Drug Metabolising Enzyme System

(Patrick Creaven, Larry Allen and Carole Williams)

In interaction of thalicarpine with microsomal aniline hydroxylase system, studied in rat liver microsomes, it was found that thalicarpine was a powerful inhibitor of aniline hydroxylase (concentration of 0.11 μM of thalicarpine producing a 30% inhibition at an aniline concentration of 2.0 μM. The inhibition was of the mixed type. Pretreatment of rats with thalicarpine 35 mg/kg Q12H X 3 did not effect aniline hydroxylase activity, microsomal cytochrome P450, cyctochrome b_5, microsomal RNA of nuclear DNA. Pretreatment with thalicarpine and methylcholanthrene, a powerful microsomal enzyme inducer, leads to the inhibition by thalicarpine of the induction in aniline hydroxylase activity, microsomal RNA and nuclear DNA normally seen with methylcholanthrene. The increase in cytochrome P_{450} and cyctochrome b_5 produced by methylcholanthrene was not affected by concomitant pretreatment with thalicarpine. The ability thalicarpine to prevent the induction by methylcholanthrene of aniline hydroxylase activity cannot therefor be due to a prevention of cytochrome P450 biosynthesis and must be due to an alteration at some prior point in the microsomal electron transport chain or to false coding producing a catalytically inactive cytochrome P_{450}. These results are being integrated with studies on the mode of action of thalicarpine in an attempt to gain more insight into the activity of thalicarpine at the subcellular level. The project continues.
(Allen and Creaven, 1973, 1973)

Stage V, 5XX-Ic LA:

Phase II Studies in the Treatment of Lung Cancer

(Martin Cohen, Robert Kane, Robert Diasio, Barry Strauss, Augustin Schwartz and medical staff)

The following Phase II Studies were established by cell type:

		with progression
Epidermoid	VP 16, 290 mg/m^2 i.v. q w →	streptonigran, 1 mg/m^2 p.o. biw
Large cell	streptonigran 1 mg/m^2 p.o. biw →	VP 16, 290 mg/m^2 q w
Small cell	emetine, 75 mg/m^2 i.v. q w →	6TG, 75 mg/m^2 p.o. q d

Adenocarcinoma 6TG, 75 mg/m^2 p.o. q d → emetine, 75 mg/m^2 i.v. q w

The above protocol has just begun to accrue patients.

Stage V, 5XX-Ih LA:

Initial Chemotherapeutic Trial in Patients with Inoperable or Recurrent Cancer of the Prostate
(Francisco Tejada, John Bresette WVAH), Richard Simon, Kenneth Cacks and medical staff)

A controlled prospective study was initiated to evaluate the response to chemotherapy of patients with Stage D adenocarcinoma of the prostate in two groups: I, patients with no prior hormonal manipulation are randomized to receive DES+5-FU vs DES alone; II, patients with prior hormonal manipulation and progression are randomized to receive either 5-FU or CCNU with crossover at progression. To date, 7 patients have been entered with 3 too soon to evaluate. Four are evaluable, with the following results:

Group I:	DES+5-FU:	1 pt. At 1 month, subjective response - performance change from 3 to 1. Peripheral blood acid phosphatase from 13.3 to 9.4 Discharged home, died overnight, no post.
	DES alone:	1 pt. At 4 months, partial response with change peripheral blood acid phosphatase from 42 B.U. to 19 B.U.
Group II:	5-FU:	1 pt. At 2 months, no change, then progression, crossover.
	CCNU:	1 pt. At 4 months, partial response with histologically positive nodes from 1 X 1 cm to non-palpable. Improvement in performance and weight gain of 20 lbs.

So far the patients are tolerating the chemotherapy with acceptable degree of toxicity to the initial dose of agents. The initial accrual of patients is somewhat slow; measures are being taken to improve the accrual rate.

Stage V, 5XX-m LA:

Phase II Study for Treatment of Hepatoma with Adriamycin

(Robert Kane, Robert Diasio and medical staff)

Because of the availability of patients with hepatoma in the VA system and the preliminary data on adriamycin activity, a Phase II adriamycin protocol has been designed for patients with hepatoma. Two patients have been entered thus far, one with transient response, the other currently responding.

Stage V, 5xx-III LA:

Calcitonin Study (Marker Substances Laboratory)

(Aron Primack, Omega Silva, Elaine Bild, Brenda Shelton and Jeffrey Lee)

The study consists of taking blood samples from Oncology patients. These samples are assayed to observe changes in calcitonin levels with changes in treatment, etc. The Chago cells have been used in tissue culture, assaying their supernatant to see if they produce calcitonin. Currently excess amounts of calcium and no calcium are being added to the media to observe the amounts of calcitonin produced.

Marker Substances in Patients with Lung Cancer

(Aron Primack and Elaine Bild)

Follow-up of various marker substances in lung cancer patients: CEA, AFP, hPL, PAP. There was a beginning trend toward follow-up of markers as a means of following disease activity. Forty patients with lung cancer were studied. Blood samples were taken every two weeks on each patient.

Cell Culture and Drug Effects

(Aron Primack and Elaine Bild)

The effects of various antineoplastic agents on growth of lung cancer cells in culture and the effects of these drugs on hormone production by the cancer cells were studied. At the end of the reporting period there were obvious differences noted among drugs used in both growth and hormone production. There was also evidence that when actinomycin D was used, hormone production was not necessarily a good marker system. The study continues.

Stage V, 5XX-V LA:

Evaluation of Serum and Bone Marrow Acid Phosphatase and $TC^{99}m$ Bone Scan as Criteria for Response in Prostatic Cancer

(Francisco Tejada and Brenda Shelton)

In conjunction with the Phase II Study for Treatment of Patients with Cancer of the Prostate, this project was designed to evaluate response. Since normal values for bone marrow acid phosphatase levels have not been established, the values were studied in patients with malignancies not primary to the bone or prostate and without bone or bone marrow metastases, documented by bone surveys, bone scan, marrow aspirate, and biopsy. So far 6 patients have been studied: peripheral acid phosphatase ranged from 1.0 to 2.1, using the Babson method; the bone marrow acid phosphatase ranged from 0.7 to 4.71. One patient with metastatic small cell carcinoma of the lung to the bone marrow had a peripheral blood acid phosphatase of 1.0 and a bone marrow acid phosphatase of 6.2, being the normal peripheral blood values of the Technic < 5.0 U.B. Of 7 patients entered into the Phase II study, bone marrow aspirates were obtained from 6 patients at time of staging. One had acid phosphatase < 5.0 U.B. in blood and marrow, while having obvious metastatic disease in the bone; 3 patients had > 5.0 U.B. in blood and marrow; and 2 patients had < 5.0 U.B. in blood and > 5.0 U.B. in marrow. These results are interesting; the study continues with follow-up samples also.

Stage V, 5XX-V LA:

Fiberoptic Bronchoscopy

(Martin Cohen, Robert Kane, Anita Tvardzik and Charlotte Williams)

One hundred and thirty bronchoscopies were performed for diagnostic and therapeutic reasons, and for follow-up of lesions in patients. Studies include:

1. biopsies after intravenous thymidine,
2. bacteriologic evaluation of lower respiratory tract,
3. cytologic changes in tumor cells, and
4. effect of bronchoscopy on blood cultures.

Fiberoptic Peritoneoscopy

(Francisco Tejada and Aron Primack)

The primary aims of the procedure are to: 1) directly view the surface of the liver, and 2) obtain biopsies of suspicious areas under direct visualization. The procedure is being used now as standard diagnostic approach for staging of patients with small cell carcinoma and adenocarcinoma of the lung who have no other evidence for distant metastases and for staging of patients with malignant lymphomas. Of 14 patients submitted to peritoneoscopy for no metastatic disease, 4 did have positive liver involvement. A prospective study will be conducted of patients with Stage C & D adenocarcinoma of the prostate to see the value of peritoneoscopy and liver biopsy in staging and follow-up after treatment.

Stage VI, 6X1-Ic LA:

Intensive Chemotherapy and Radiotherapy in Patients with Bronchogenic Carcinoma

(Oleg Selawry and medical staff)

In cooperation with the Mayo Clinic and the University of Capetown, South Africa, 405 patients with untreated inoperable lung cancer were staged as having limited disease (1 hemithorax ± ipsilateral cervical lymph nodes) or extensive disease. Patients with limited disease were randomized to either radiotherapy alone (5000 R - split course) or radiotherapy plus combination chemotherapy (alkylating agent + methotrexate). Patients with extensive disease were randomized to either Regimen A (MTX 20 mg/m^2 twice weekly p.o. + CYT 1100 mg/m^2 i.v. q 3 weeks for small cell carcinoma and adenocarcinoma or MTX + mechlorethamine 15 mg/m^2 i.v. q 3 weeks for epidermoid carcinoma and large cell carcinoma; or Regimen B: MTX 10 mg/m^2 i.v. twice weekly p.o. + CCNU 50 mg/m^2 q 6 weeks p.o. + CYT 50 mg/m^2 i.v. q 3 weeks (small cell and adenocarcinoma) or MTX + CCNU + HN2 10 mg/m^2 i.v. q 3 weeks (epidermoid, large cell carcinoma). Upon progression, chemotherapy was continued with CCNU + OH-urea and at subsequent progression, procarbazine + vincristine for Regimen A, and vincristine + procarbazine + OH-urea for Regimen B. Results: For limited disease (187 patients), adjuvant chemotherapy was ineffective. For extensive disease (218 patients), Regimen B prolonged median survival significantly for small cell (250 days vs 170 days) and for adenocarcinoma (240 days vs 135 days) due to longer response to the first drug combination. Objective response was 31% and 6% in Regimen A, 57% and 30% in Regimen B for small cell and adenocarcinoma, respectively. No advantage of B over A occurred for epidermoid carcinoma and large cell carcinoma. It was concluded that 3-drug chemotherapy is superior to 2-drug chemotherapy in extensive small cell and adenocarcinoma of the lung.

Stage VI, 6X1-Ij, k LA:

Participation in Cooperative Studies: ALGB

(Francisco Tejada, Mary J. Bettis and medical staff)

Combination Chemotherapy of Stage III and IV Lymphosarcoma and Reticulum Cell Sarcoma, Induction and Maintence (2 patients).

Treatment of Advanced Lymphosarcoma and Reticulum Cell Sarcoma with Adriamycin as Induction and Maintenance with Procarbazine or Procarbazine + CCNU (1 patient)

Comparison of Daunorubicin (CNR) alone with DNR + Ara-C +TG and Ara-C + TG Induction Therapy of Previously Untreated AML (1 patient)

Prevention of Blast Crisis in CML by Pulse Doses of CCNU and Ara-C During the Course of Busulfan Maintained Remission (1 patient)

Comparison of Triple Alkylating Agents (L-PAM/Cyt/BCNU + Pred) with Single Alkylating Agent (L-PAM) + Pred. for Primary Treatment of Multiple Myeloma (2 patients)

Stage VI, 6X1, Im LA:

Participation in Cooperative Studies: ECOG
(Martin Cohen)

Comparison of Methotrexate with Leukovorin versus Methotrexate Alone, Plus Leukovorin, Cytoxan, and Ara-C in Patients with Epidermoid Carcinoma of the Head and Neck (2 patients)

Phase III Study of Adriamycin versus Cycloleucine in the Treatment of Sarcomas (1 patient)

Stage VI, 6X1-Ic, 6X2-II; 6X1-Im, 6X2-II LA:

Growth Characteristics of Solid Tumors as Determined by in vivo Labelling with ^{3}HTdR
(Marc Straus, Francisco Tejada and Susan Krezoski)

Lung Cancer: Nine patients with advanced lung cancer were injected with .2mCi/kg ^{3}HTdR i.v. A series of experiments are being conducted to determine the most efficient, least traumatic, and most reproducible approaches to obtain tumor samples for cell kinetic analysis. Forty time aspirates were taken from each of 3 metastatic nodules of 1 patient with large cell anaplastic carcinoma; the PLM curves were compared. Nine bronchoscopic tumor biopsies were taken in succession from 1 patient with small cell carcinoma; the labelling index was determined on each. In general, a large variation in growth parameters are found in a given mass and it is necessary to remove multiple aspirates or biopsies for representative data. The data thus obtained will be compared for each cell type and stage of disease before and after treatment. The initial emphasis will be on epidermoid carcinoma of the lung. Tumor will be obtained by thoracotomy, bronchoscopy, or aspiration.
Head and Neck Epidermoid Carcinoma: In 3 patients deemed not curable by surgery or radiotherapy, ^{3}HTdR was injected as above. Samples of tumor were obtained by needle aspirate and/or punch or forceps biopsy at approximately

3-4 hr intervals for 72-96 hrs. Methotrexate was then administered as a single oral dose of 30 mg/m^2 and sampling of tumor continued for an additional 3-4 days. Standard radioautographic methods were employed and the data are now being analyzed. It appears that the dose of ^{3}HTdR is adequate for determining PLM curves. Other questions to be resolved include:

a) comparison of kinetic data obtaining simultaneously by aspirates and biopsies,
b) the effect of methotrexate in the cell cycle,
c) comparison of normal and malignant PLM data in the same patient, and
d) comparison of primary and metastatic PLM data in the same patient.

Stage VI, 6X1-IV LA:

Laminar Air Flow Reverse Isolation Project
(Martin Cohen, Byron Fossieck, Anita Tvardzik and Charlotte Williams)

Entered into the protocol were:
8 patients, Group I: 6 small cell carcinoma, 1 adenocarcinoma, 1 epidermoid
3 patients, Group II: 1 small cell carcinoma, 1 epidermoid, 1 adenocarcinoma
6 patients, Group III: 6 small cell carcinoma
Increase in dosage after little toxicity was observed, the dosage levels now are:

CCNU	100 mg/m^2 q 6 w
CTX	1000 mg/m^2 q 3 w
MTX	15 mg/m^2 biw

Response rate for the small cell Group I: 66%

Stage VI, 6X1-VI (50%); Stage VII, 7XX-III (50%) LA:

Quantitative Evaluation of Radio-Opacities on Chest X-Rays
(Paul Hamosh)

The evaluation of pathological chest x-rays is usually based on visual observation and therefore a "judgment" process. Automation of such evaluation could therefore be a distinctive advantage because of elimination of observer bias. We applied an image-processing device to the analysis of chest x-rays. A photo-multiplier tube is scanning a 35 mm negative of the x-ray and dividing the image into a raster ("grid") of 275x360 discreet points. Each point has an assigned gray level value from 0-15. The image is reconstituted by an IBM 360-44 computer which can be programmed to analyze the image. We have evolved a number of methods of interpreting x-rays both for a "texture analysis" and solid lesions. At this point it can be concluded that the computer method has no advantage over other planimetric approaches. The major "hardware" problem is the limitation to sixteen gray levels. An expansion to 128 levels is possible, but needs a computer memory in excess of the available. Recently, this approach was rendered obsolete by the development of the "ACTA" (Automatic Computerized Transverse Axial) tomographic scanner. This method combines the process of x-ray taking with the computer analysis. Presently, we are engaged in pilot studies of applying this method to the chest.

Stage VII, 7XX, Ic LA:

Participation in Cooperative Studies: Working Party for Therapy of Lung Cancer

Chemotherapy of Extensive Epidermoid Carcinoma of the Lung (Marc Straus and medical staff) (8 patients)

Radiotherapy of Regional Epidermoid Carcinoma of the Lung (Robert Andrews, Marc Straus and medical staff) (8 patients)

Intensive Chemotherapy and Radiotherapy of Small Cell Carcinoma of the Lung (Martin Cohen and medical staff) (Regional Disease: 3 patients; Extensive Disease: 4 patients)

Therapy of Limited Extent Adenocarcinoma and Large Cell Carcinoma of the Lung (Lawrence Broder, Robert Andrews and medical staff) (8 patients)

Cancer Biology and Diagnosis LA:

Study of Problems in Morphology and Behavior of Lung Cancer by Cell Type (Mary Matthews and Suryabala Kanhouwa)

A retrospective study of 418 patients with lung cancer, autopsied over a 10-year period at the VA Hospital, Washington, D.C., was undertaken to assess the validity of the lung cancer classification prior to its use by the Working Party for Therapy of Lung Cancer and to determine if the classification could provide preliminary information concerning behavior of lung cancer according to cell type. From this study, it would appear that moderately differentiated tumors, in general, are less aggressive than their poorly differentiated counterparts. Well and moderately differentiated epidermoid carcinomas tend to be localized to the thorax (55%) and metastasize to the liver and kidneys in less than ¼ of the cases. Metastases to other organ sites is even less frequent. Well and moderately differentiated adenocarcinomas, to the contrary, metastasize frequently and predictably to the adrenals, liver, bone, kidney and central nervous system. Anaplastic tumors (large and small cell types) and poorly differentiated tumors with precominant anaplastic or giant cell components metastasize widely and to unlikely sites. The majority of small cell tumors metastasize to the liver, adrenals, bone, central nervous system, pancreas, and endocrine organs. Anaplastic large cell tumors metastasize in a pattern reminiscent of adenocarcinomas. Poorly differentiated epidermoid tumors and tumors with prominent giant cell components metastasize with exceptional frequency to the mucosa and submucosa of the gastrointestinal tract. (Matthews and Kanhouwa, 1974, 1974)

Incidence of Bronchial Epithelial Changes and Carcinoma in situ in the Tracheobronchial Tree of Lung Cancer Patients

(Mary Matthews and Suryabala Kanhouwa)

At the time of autopsy on patients on protocols of the Working Party for Therapy of Lung Cancer, the tracheobronchial tree and all broncho-pulmonary segments are dissected, labeled separately and submitted for microscopic examination. Preliminary findings show chronic bronchitis to be a constant, reserve cell hyperplasia is seen frequently with small cell carcinoma, squamous metaplasia (regular and atypical) is seen more commonly with squamous cell carcinoma, squamous dysplasia of the bronchial epithelium was present in 3 cases of small cell carcinoma treated in the LAF units, in a few cases *in situ* was seen.

Electron Microscopic Study of Human Lung Cancer

(Mary Matthews and Suryabala Kanhouwa)

All available tissues on lung tumors (biopsies or surgically resected lung) are studied by electron microscopy and classified according to the WPL classification. Observations: Squamous cell carcinomas have shown desmosomes, tonofilaments, and keratokyaline granules. Microvilli and cilia have been noted in adenocarcinoma. Neuro-secretory granules have been observed in small cell tumors. Virus-like particles were identified in one case of adenocarcinoma.

Electron microscopy complementing light microscopy is of value in classification of some lung tumors.

Cytologic Typing of Lung Cancer

(Mary Matthews and Suryabala Kanhouwa)

All positive cytologic specimens are classified according to the WPL classification into 4 major types of lung carcinoma. The cytologic diagnoses of lung cancer are then compared with histologic diagnoses. Results of a one-year study on 111 patients with lung cancer show 100% consistency between the cytological and histological diagnosis for well and moderately well differentiated adenocarcinoma and epidermoid carcinoma, 90% for small cell carcinoma, and 83% for large cell anaplastic carcinoma. The poorly differentiated adenocarcinoma and epidermoid carcinoma show very poor correlation between cytologic and histologic diagnosis. Overall consistency with histologic diagnosis was 77.2%.

Stage VIII, 8XX:

I. Administration of the NCI-VA Medical Oncology Branch must conform to procedures and regulations of two agencies: The Veterans Administration and the National Cancer Institute. Where possible the requirements of administrative procedures are combined to save time and effort. The administrative assistant to the Branch Chief acts as basic liaison in administrative matters, referring necessary problems to the Administrative Officer of the VA Hospital or the Administrative Officer at the VA Central Office; or to the Administrative Officer, MO, DCT, or the Administrative Office, DCT, NCI.

II. Dissemination of Information and Training

Medical Staff

Rounds

1. Weekly Ward Walk Rounds. All patients on oncology ward were seen by 2 attendings, 1 or more other oncology senior staff and all oncology trainees, including clinical associates, oncology fellows, medical interns, and residents on oncology electives.

2. Weekly Grand Rounds. Multidisciplinary approach to oncology with medical oncologists, radiotherapists, pathologists, and surgeons, where indicated, discuss the management and therapy of certain selected patients from the oncology ward or outpatient clinics. During these rounds, selected patients are seen by the entire oncology staff.

3. Washington Hospital Center. One fellow has been assigned to the Oncology Service of WHC on a monthly rotation to provide consultation to patients on that service and also throughout the hospital. Weekly rounds have been made by the entire junior staff and 2 & 3 members of the senior staff on selected oncology patients.

4. Consult Rounds. All patients in the Washington VA Hospital on whom consultations are requested are discussed and seen during weekly consult rounds. Rounds are made by 2 or more senior staff physicians and the junior staff assigned to the consultation service.

5. Morning Report. All physicians, social worker, pharmacist and head nurse on the Oncology Branch attend morning report. During these sessions any patient-related problems of the previous day and any new admissions or deaths are discussed.

6. Autopsy Review. All autopsies performed on patients on the Oncology Service are reviewed with the Pathology Service on the day the autopsy is done. All oncology physicians attend.

Conferences

1. Oncology Basic Science Lectures. Over the past year, 21 lectures have been given on topics related to oncology. Outside guest speakers have given 5 of these lectures with the remaining lectures given by the Oncology Senior Staff.

2. Oncology Clinical Lectures. Twenty-eight oncology lectures have been given over the past year. These lectures have been organ site directed and multidisciplinary with medical oncologists, radiotherapists, pathologists and surgeons participating. A syllabus of these lectures is in preparation for use in our training program in medical oncology.

3. Lectures to the VA Hospital Medical Service. The Oncology Senior Staff is responsible to the Department of Medicine for giving 10 lectures on various aspects of medical oncology to the house staff and senior staff of the Medical Service during the year.

4. Participation in Medical Grand Rounds. The Oncology Branch has taken part in 3 VA Hospital Medical Grand Rounds during the past year.

5. Staging Conference. On a weekly basis, the senior and junior Oncology staff reviews patients either going onto or coming off protocol studies when there are difficult decisions to be made on patient management. This conference provides a forum for the views of members of the Oncology staff on these complex problems. The Radiation Therapy Department also attends these conferences so that joint decisions regarding radiation therapy can be made.

6. Joint NCI-VA Medical Oncology Conference. The NCI-VA Medical Oncology Branch is responsible at monthly intervals for organizing a clinical conference at the National Cancer Institute as part of the joint training program.

Training Program

1. Postgraduate

Twelve interns (rotating 2 at a time for 8-week periods)
Five clinical associates present for 9 months each
Seven clinical associates present for 3 months each
Two research associates present for 6 months each
One medical oncology fellow present for 12 months
Four medical fellows on 1 month elective each

2. Other Training -

One medical student for 4 months
Twelve 3rd-year George Washington University medical students received training in physical diagnosis on the Oncology Ward

Medical Attendingships. All senior staff members serve 1 to 2 months a year as attendings on the general medical wards of the VA Hospital.

Lectures and Abstracts

A. Larry M. Allen, Ph.D.

1. "Plasma Levels and Urinary Excretion of Isophosphamide, an Antineoplastic Cyclophosphamide Analog," presented at the 15th National Meeting of the APhA Academy of Pharmaceutical Sciences, San Diego, California, November, 1973, Abstracts of the APhA Academy of Pharmaceutical Sciences. 15:97, 1973.

2. Allen, L.M., and Creaven, P.J. Inhibition of Macromoleculab Biosynthesis in Cultured L1210 Mouse Leukemia Cells by Thalicarpine (NSC 68075). Cancer Res. 33:3116, 1973. (Abstract)

3. Allen, L.M., and Creaven, P.J. Inhibition of DNA, RNA and Protein Biosynthesis in Cultured L140 Cells by Thalicarpine (NSC 68075). Cancer Res. 33:3112, 1973. (Abstract)

4. Accepted for membership in the American Association for Cancer Research, April, 1974.

5. Active membership and participation in: American Chemical Society.

B. Lawrence E. Broder, M.D.

1. "Chemotherapy of Malignant Insulinomas with Streptozotocin," presented at the Eighth International Congress on Diabetes, Brussels, Belgium, July, 1973. Proceedings, Excerpta Medica, 1974.

2. "Pancreatic Islet Cell Carcinoma," presented at Georgetown University Hospital, Washington, D.C., August, 1973.

3. Certified by the American Board of Internal Medicine, September, 1973.

4. "Therapy of Retinoblastoma," presented at the Symposium on Pediatric Neoplasma, Bethesda, Md., November, 1973.

5. Appointed Associate Acting Executive Director for the International Society for Study of Lung Cancer, January, 1974.

6. "The Value of Pharmacokinetic Data in the Design of Clinical Trials in Breast Carcinoma," presented at the Meeting of the American Society of Clinical Oncology, March, 1974. Proc. ASCO-AACR, 15:829, 1974. (Abstract)

7. "Chemotherapy in Treatment of Lung Cancer," presented at a course sponsored by the American College of Physicians, Albany, N.Y., June, 1974.

8. Accepted to membership in:
   ECOG
   ALGB
   Working Party for Therapy of Lung Cancer
   International Association for Study of Lung Cancer

C. Martin H. Cohen, M.D.

1. Appointed Chief, Clinical Section, NCI-VA Medical Oncology Service, September, 1973.

2. Appointed Assistant Chief, NCI-VA Medical Oncology Service, September, 1973.

3. Appointed Chairman, Chemotherapy Committee of the Radiation Therapy Oncology Group, September, 1973.

4. "The Laminar Air Flow Protective Environment," presented at the meeting of the Association of Military Surgeons of the U.S., Washington, D.C., November, 1973.

5. "Eastern Cooperative Oncology Group Lung and Sarcoma Studies," presented at the Phase I - II Liaison Committee, Bethesda, Maryland, December, 1973.

6. "Bleomycin Studies in the ECOG," presented at the New Drug Seminar, Bethesda, Maryland, February, 1974.

7. Active membership and participation:
   American Society of Hematology
   Society of Experimental Hematology
   American Association for Cancer Research
   American Society of Clinical Oncology
   Acute Leukemia Group B
   Education Committee, NCI
   Contract Review Committee, NCI
   Eastern Cooperative Oncology Group (Executive Officer)
   Working Party for Therapy of Lung Cancer
   International Society for Study of Lung Cancer

D. Patrick J. Creaven, M.D., Ph.D.

1. "The Application of Screening to the Initial Clinical Laboratories of New Anticancer Drugs," presented at the 5th Conjoint Meeting, Annapolis, Maryland, July, 1973.

2. Appointed member of the Research and Education Committee, VA Hospital, July, 1973.

3. "Phase I Study of Thalicarpine," presented at the Phase I Working Group Meeting, Bethesda, Maryland, June, 1973. Minutes of Phase I Working Group, p. 22-25.

4. Appointed Chief, Oncologic Pharmacology Section, NCI-VA Medical Oncology Service, September, 1973.

5. "Current Status of Ongoing Phase I Studies: Thalicarpine," and "VP-16," presented at the Phase I Working Group Meeting, Bethesda, Maryland, October, 1973. Minutes of the Phase I Meeting, p.156, October, 1973.

6. "Clinical Studies and Pharmacology of ICRF-159," presented at the Meeting on Current Status of ICRF-159, Bethesda, Maryland, November, 1973. Minutes of the ICRF Meeting, November, 1973.

7. Phase I Studies: "VP-16," and "Thalicarpine," presented at the New Drug Liaison Meeting, December, 1973. Minutes of the New Drug Liaison Meeting, p. 139-140, December, 1973.

8. "Bioavailability of the Antineoplastic Drug ICRF-159: Relation to Clinical Toxicity" and "Thalicarpine Interaction with the Microsomal Drug Metabolizing System," presented at the 15th National Meeting of the APhA Academy of Pharmaceutical Sciences, San Diego, California, November, 1973. Abstracts of the APhA Academy of Pharmaceutical Sciences Meeting, 15:96, 1973.

9. "Thalicarpine Interaction with the Microsomal Drug Metabolizing Enzyme System," presented at the 15th National Meeting of the APhA Academy of Pharmaceutical Sciences, San Diego, California, November, 1973. Abstracts of the APhA Academy of Pharmaceutical Sciences Meeting, 15:95, 1973.

10. "Phase I: Thalicarpine," presented at the Meeting of the Phase I Working Group, Bethesda, Maryland, January, 1974.

11. Creaven, P.J., Cohen, M.H., Hansen, H.H., Selawry, O.S. and Taylor, S.G. Phase I Clinical Trial of a Single Dose and 2 Weekly Schedules of ICRF-159 (NSC 129943). Cancer Chemother. Rep. 58:3, 1974.

12. Newman, Stephen T., Creaven, P.J., and Selawry, O.S. Phase I Trial of 4'-demethylepipodophyllotoxin 9-(4,6-O-ethylidene β-d-glucopyranoside) (NSC 141540, VP 16-213). Proc. ASCO-AACR, 15:132, 1974. (Abstract)

13. "Phase I Study of Thalicarpine," presented at the Phase I Working Group, Bethesda, Maryland, May, 1974.

14. Site Visits for Phase I Contracts to:
    Emory University, April, 1974.
    Boston Children's Hospital, May, 1974.

15. Accepted for membership in:
    International Society for Study of Lung Cancer, March, 1974.
    American Association for Cancer Research, April, 1974.

16. Active membership and participation in:
    American Chemical Society
    Biochemical Society
    New York Academy of Sciences
    American Association for the Advancement of Science
    Academy of Pharmaceutical Sciences
    American Federation for Clinical Research, Secretary-Treasurer, D.C. Chapter
    Acute Leukemia Group B
    Eastern Cooperative Oncology Group
    Working Party for Therapy of Lung Cancer

E. Byron E. Fossieck, M.D.

1. Appointed Assistant Professor of Medicine, Department of Medicine, George Washington University Medical Center, Washington, D.C. July, 1973.

2. "Newer Diagnostic Methods in Infectious Diseases," presented at the VA Hospital House Staff Conference, VA Hospital, Washington, D.C., November, 1973.

3. "Neoplasms and Infection," presented at the VA Hospital Staff Conference, VA Hospital, Washington, D.C., February, 1974.

4. "Salmonellosis," presented at the VA Hospital House Staff Conference, VA Hospital, Washington, D.C., May, 1974.

F. Suryabala Kanhouwa, M.D.

1. "Cytologic Typing of Cancer," presented at the Meeting of the Working Party for Therapy of Lung Cancer, Bethesda, Maryland, November, 1973.

2. Physicians Recognition Award 73-76, from the American Medical Association, January, 1974.

3. "Cytologic Typing of Lung Cancer," presented at a Seminar, Department of Medicine, George Washington University Medical Center, Washington, D.C., January, 1974.

4. Accepted for membership in the International Society for Study of Lung Cancer.

5. Active membership and participation in:
Washington Society of Pathologists
American Medical Association
Tropical Diseases Society
American Society of Clinical Pathologists
American Board of Pathology
Working Party for Therapy of Lung Cancer

G. Mary J. Matthews, M.D.

1. Appointed Chairman for Local Arrangements Committee, Combined Washington and Maryland Societies of Pathology, September, 1973.

2. "Results of Prospective Study of Lung Cancer Patients, Utilizing WPL Classification in the Evaluation of Diagnosis and Behavior of Tumor According to Cell Type," presented at the Meeting of the Working Party for Therapy of Lung Cancer, Bethesda, Maryland, November, 1973.

3. Appointed Co-Chairman of the Pathology Subcommittee Working Party for Therapy of Lung Cancer, Bethesda, Maryland, November, 1973.

4. "Lipase Secreting Acinar Cell Carcinoma of the Pancreas, a Clinical-Pathologic Electron Microscopic and Biochemical Study," presented at the Pathology Seminar, George Washington University Medical Center, Washington, D.C., February, 1974.

5. "Functional Acinar Cell Carcinoma, a Clinico-Pathologic and Electron Microscopic Study and Correlation," presented at the Pathology Seminar, George Washington University Medical Center, Washington, D.C., March, 1974.

6. Accepted for membership in:
International Society for Study of Lung Cancer

7. Active membership and participation in:
American Society of Cytology
American Medical Association
International Academy of Pathologists
American Association for Advancement of Science
American Medical Women's Association
Working Party for Therapy of Lung Cancer. (Co-Chairman of the Pathology Committee)
Washington Society of Pathologists
American Association of Pathologists and Bacteriologists
International Academy of Pathology

H. Aron Primack, M.D.

1. Appointed Associate Acting Executive Director for the International Society for the Study of Lung Cancer, September, 1973.

2. "Correlation of AFP with Treatment in Hepatocellular Carcinoma," presented at the Third Conference and Workshop on Embryonic and Fetal Antigens in Cancer, Knoxville, Tennessee, November, 1973.

3. "Staging of Hepatocellular Carcinoma," presented at the D.C. Chapter of the AFCR Meeting, Washington, D.C., February, 1973.

4. Becker, K.L., Matthews, M.J., et al. Prospective Study of the Gynecomastia Associated with Hyperthyroidism. Fed. Proc. Exper. Path., 32:216, 1973.

5. Burnes, W.A., Vanderweide, T., and Matthews, M.J. Functional Acinar Cell Carcinoma of the Pancreas. Proc. Electron Microscopy Soc. Amer., 1973.

6. Active membership and participation in:
   American Society for Clinical Oncology
   American Association for Cancer Research
   American Federation for Cancer Research
   Working Party for Therapy of Lung Cancer
   International Society for Study of Lung Cancer

I. Oleg S. Selawry, M.D.

1. "Advances in the Treatment of Lung Cancer," presented at the Harvard University School of Medicine Seminar, Boston, Mass., August, 1973.

2. Appointed by the Executive Committee of the National Cancer Institute to coordinate the National Lung Cancer Program, September, 1973.

3. "Current Approaches to Treatment of Lung Cancer," presented at a Seminar, University of Sothern California Medical School, Los Angeles, California, October, 1973.

4. "Current Status of Ongoing Phase I Studies." "Thalicarpine" and "VP-16," presented at the Phase I Working Group Meeting, Bethesda, Maryland, October, 1973.

5. "Introduction to and Status of Planning for the National Lung Cancer Program," presented at the Meeting of the National Cancer Advisory Board, Bethesda, Maryland.

6. "Report on Status of Current Research in Lung Cancer," presented at the Meeting of the Working Party for Therapy of Lung Cancer, November, 1973.

7. Elected Acting Executive Director, International Society for the Study of Lung Cancer, November, 1973.

8. "Chemotherapy of Lung Cancer," presented at Medical Grand Rounds, Memorial Sloan-Kettering Cancer Center, New York, New York, January, 1974.

9. "Phase I Study of Bleomycin in the United States," presented at the New Drug Seminar, Bethesda, Maryland, February, 1974.

10. "Overview" and "Chemotherapy," for the Session on Carcinoma of the Lung, presented at the Seminar on Modern Concepts in Oncology, sponsored by the University of California at Los Angeles School of Medicine, Indian Wells, California, March, 1974.

11. Participation in the First Joint US-USSR Meeting for Treatment of Lung Cancer and Annual Review Meeting for Chemotherapy, Moscow, USSR, March 3-15, 1974. Lectures given: "Consideration for chemotherapy and

Multidisciplinary Treatment of Lung Cancer," "Study Design and Background Data for Multidisciplinary Treatment of Small Cell Carcinoma," presented at the IECO, Moscow, USSR.

Participation in Discussions on Cooperation in Areas of Research in Treatment of Lung Cancer.

Appointed to Serve as Focal Point for Communications Between the USSR and US for all Scientific Exchange in the Area of Lung Cancer.

12. "Chemotherapy as Adjuvant to Surgery for Treatment of Bronchogenic Carcinoma," presented at the XIX International Biennial Congress of the International College of Surgeons, Lima, Peru, March, 1974.

13. "Current Status of Bronchogenic Carcinoma," presented at the Pulmonary Conference, Georgetown University Hospital, Washington, D.C., April, 1974.

14. "Prognosis Factors and Criteria of Response in Lung Cancer," presented at the Symposium on Clinical Pharmacology, EORTC, Institut Jules Bordet, Brussels, Belgium, May, 1974.

15. "Role of Chemotherapy in Treatment of Lung Cancer," presented at the Meeting of the American Osteopathic Association, Bethesda, Maryland, June, 1974.

16. "Phase II Studies of Chemotherapeutic Agents in Prostatic Carcinoma," presented at the Workshop on Prostatic Cancer National Prostatic Cancer Project, Buffalo, New York, June, 1974.

17. Active membership and participation in:
    American Association for Cancer Research
    American Federation for Clinical Research
    New York Academy of Science
    American Society of Clinical Oncology
    American Society of Hematology
    American Federation of Clinical Oncologic Societies
    Acute Leukemia Group B
    Eastern Cooperative Oncology Group
    NCI Operations Committee, NCI
    Decision Network Committee, NCI
    Experimental Design Committee, NCI
    Specialty Fellowship Board, NCI
    Veterans Administration Merit Review Board for Oncology
    Working Party for Therapy of Lung Cancer (Chairman)
    International Society for Study of Lung Cancer

J. Marc Straus, M.D.

1. "Superior Vena Cava Syndrome - Diagnosis and Treatment," presented at Grand Rounds, Boston University Hospital, Boston, Mass., December, 1973.

2. "Cell Biology of the Design of Cancer Chemotherapy," presented at Rounds, Edward Mallinckrodt Institute of Radiology, St. Louis, Missouri, January, 1974.

3. "Uptake and Excretion of Tritiated Thymidine in Man," Cell Kinetics Rounds, Division of Cancer Treatment, NCI, Bethesda, Maryland, January, 1974.

4. "Analyzing the Growth of Solid Tumors," NCI-VA Medical Oncology Grand Rounds, VA Hospital, Washington, D.C., February, 1974.

5. "Newer Approaches to Management of Lung Cancer," presented at Medical Grand Rounds, Suburban Hospital, Bethesda, Maryland, February, 1974.

6. "Cell Kinetics and Lung Cancer," presented at Hematology-Oncology Grand Rounds, Walter Reed Hospital, Washington, D.C., February, 1974.

7. "Kinetics of Tumor Growth," presented at Grand Rounds, VA Hospital, Washington, D.C., March, 1974.

8. Site Visits (Cell Kinetics):
   Mayo Clinic, September, 1973
   Roswell Park, October, 1973
   Ohio State University, October, 1973

9. Active membership and participation in:
   American Association for Cancer Research
   Acute Leukemia Group B
   American Federation for Clinical Research
   Working Party for Therapy of Lung Cancer (Executive Officer)
   International Society for Study of Lung Cancer

K. Francisco Tejada, M.D.

1. "Head and Neck Cancer: A Review," presented at the ENT Seminar, Surgical Service, VA Hospital, Washington, D.C., September, 1973.

2. "Prostatic Cancer: A Review," presented at a Medical Oncology Seminar, Bethesda, Maryland, November, 1973.

3. Certified by the American Board of Internal Medicine as a Diplomate in the Subspecialty of Oncology, January, 1974.

4. Certificate of Completion of the Radiation Course, by the Radiation Committee, NIH, Bethesda, Maryland, November, 1973.

5. "Endogenous Perotonin Release from Platelets, and in vivo Test for the Diagnosis of ITP," presented at the Meeting of the American Society of Hematology, Chicago, Illinois, December, 1973.

6. "Chemotherapy of Carcinoma of the Ovary" "Sterility and Infertility in Relation to the Use of Chemotherapeutic Agents," and "Use and Abuse of Laparotomy in Patients with Hodgkin's Disease and Non-Hodgkin's Lymphoma," presented at the XIX International Biennial Congress of the International College of Surgeons, Lima, Peru, March, 1974.

7. Accepted for membership in:
   ALGB
   Peruvian Medical College
   International Society for Study of Lung Cancer

8. Active membership and participation in:
   ECOG
   Working Party for Therapy of Lung Cancer

Nursing Staff

The Ward of the NCI-VA Medical Oncology Branch is used as a training agency for nursing students in their junior year from Georgetown University. Nine to ten students have utilized the Ward during the winter and spring quarters.

In-service training in the area of cancer research and care of cancer patients is carried on throughout the year for all Oncology nursing personnel.

During this year specific nurses were appointed to special responsibilities for the areas of Phase I studies, sputum cytology, Laminar Air Flow Reverse Isolation Project, bronchoscopy, peritoneoscopy, and Out-Patient Clinic. Increased effectiveness and extensive training in the use of special techniques have resulted in higher standards of performance in these areas.

Activities:

Marjorie Boyer, Head Nurse, attended the Conference on Cancer Nursing, sponsored by the American Cancer Society, Chicago, Ill., September, 1973. Anita Tvardzik, R.N., presented "The Laminar Air Flow Reverse Isolation Protective Environment" at the meeting of the Association of Military Surgeons of the U.S., Washington, D.C., November, 1973.

III. Data Processing and Support (M.J. Bettis)

The statistical assistant/chemotherapy secretary maintains records and data for all patients on study, acts as liaison for reports to cooperative research groups, randomizes patients for all studies and maintains a continuing roster of on-study and off-study patients for Phase I Study, by use of computer terminals on Wylbur System. A monthly report on specific categories for patient census is submitted to the Chief of the Branch. For the year April 1 - March 30, the data is:

Admissions: 400
Discharges: 306
Clinic Visits: 1738
Consults: 280
Deaths: 124
Total In-Patients: 180
New Patients: 130
Average Daily Patient Census: 25

Diagnoses:

Bronchogenic carcinoma	90
Head and Neck carcinoma	10
Prostate carcinoma	8
Stomach carcinoma	5
Lymphoma	4
Myeloma	4
Kidney carcinoma	4
Leukemia	3
Rectum carcinoma	3
Breast carcinoma	2
Liver carcinoma	2
Hodgkin's	2
RCSA	2
Fibrosarcoma	2
Milanoma	1
Pancreas carcinoma	1
Colon carcinoma	1
Bladder carcinoma	1

IV.

A. Research Pharmacy (Frank Pelsor, Pharm. D.)

The activities of the Research Pharmacist, NCI-VA Medical Oncology Branch, revolve around the implementation and maintenance of a unit dose distribution system. A medication profile is maintained on all patients receiving treatment at the Branch. The Research Pharmacist is responsible for procuring, preparing, and dispensing all medications used on the Nursing Unit and in the Outpatient Clinic. From January 1, 1974 to March 31, 1974, more than 325 patient profiles of continuing medication and investigational agents were prepared. A total of 2600 chemotherapy prescriptions were dispensed by the research pharmacist. In addition, the research pharmacist may participate in basic research in the pharmacology of antineoplastic compounds.

B. Oncology Hematology Laboratory (Brenda Shelton)

Aspirations	50
BMA	300
BMBX	275
CSF	100
Periph. Smears	375
Touch Prep	325
Tumor Nodule	50

Special Staining:

Slides to Histology 150
Autohemolysis test
Autoxidations of RBC's by use of H_2O
G-6-P-D
Osmotic Fragility
Acid Phosphatase Study (75)
BMA's serum whole blood serum, and bone marrow aspiration clot simultaneously prepared.
Serum Storage Bank (1000 specimens) drawn and processed in Oncology Hematology Laboratory.
Calcitonin Study in conjunction with Dr. Omega Silva (250 specimens)
CEA Study in conjunction with Dr. Waalkes (800 specimens)
Study in conjunction with Dr. Weintraub (400 specimens)
Routine Blood Drawings by the Clinical Laboratory:

Year	4656 patients
Tubes	11808

Routine Blood Drawings by Clinical Laboratory and Oncology Hematology Technologist for Out-patient Clinic:

Year	2208 patients
Tubes	4416

C. Dietetic Support (Sadie Arkwright)

The Oncology Branch dietician conducted a food habit study on thirty male patients hospitalized with lung cancer in order to establish guidelines for diet therapy. The findings revealed that there was inadequate intake of calories before and during hospitalization. The patients expressed "preference" as the main reason for choices of food prior to hospitalization and "health" the main reason for choice while hospitalized. Based on the findings, it was concluded that the food intake of patients with lung cancer can be improved if the diet is provided in a form they can most easily consume. The diet recommended: high in calories, protein, vitamin and minerals; with frequent small feedings.

Laminar Air Flow Reverse Isolation Project: New procedures were established for LAF protocol patients who will be remaining on the ward. These patients will receive the same "cooked food diet" as patients in the LAF units, but with sterilized silverware.

Activities: The dietician attended a course in "Community Nutrition Organization and Management," Howard University, 1973. The two dietary technicians attended a 6-week training program, "Advanced Nutrition," held at the VA Hospital, 1973.

D. Social Work Support (Nan Miller)

The social worker contacts all patients admitted to the Oncology Service and makes an initial screening of each to determine existing unmet needs and to anticipate problems during the patients' hospital stay and after discharge. She serves as referring agent between the patient and other departments of the hospital and outside agencies as necessary.

Publications:

1. Allen, L.M. Renal clearance of camptothecin sodium (NSC 10080): Effect of urine volume. Cancer Chemother. Rep. 57:175-184, 1973.
2. Allen, L.M. and Creaven, P.J. Activation of the antineoplastic drug isophosphamide by rat liver microsomes. J. Pharm. Pharmac. 24:585-586, 1972.
3. Allen, L.M. and Creaven, P.J. Binding of a new antitumor agent, thalicarpine (NSC 68075) to DNA. J. Pharm. Sci. 63:474-475, 1974.
4. Allen, L.M. and Creaven, P.J. Gas chromatographic method for the determination of plasma isophosphamide (NSC 109724). Cancer Chemother. Rep. 56:721-723, 1972.
5. Allen, L.M. and Creaven, P.J. Inhibition of macromolecular biosynthesis in cultured L1210 mouse leukemia cell by thalicarpine (NSC 68075). Cancer Res. 33:3112-3116, 1973.
6. Allen, L.M. and Creaven, P.J. In vitro activation of isophosphamide (NSC 109724), a new oxazaphosphorine by rat liver microsomes. Cancer Chemother. Rep. 56:603-610, 1972.
7. Allen, L.M. and Creaven, P.J. On the interaction of mechlorethamine and isophosphamide with bovine serum albumin and rat liver microsomes. J. Pharm. Sci. 62:854-856, 1973.
8. Allen, L.M. and Creaven, P.J. The effect of microsomal activation on interaction between isophosphamide and DNA. J. Pharm. Sci. 61:2009-2011, 1972.
9. Allen, L.M., Creaven, P.J. and Williams, C. Kinetics of rabbit liver uridine diphosphate glucuronyltransferase and the effect of thalicarpine inhibition. Biochem. Pharmacol. (In press)

10. Broder, L.E. and Carter, S.K. Pancreatic islet cell carcinoma. I. Clinical features of 52 patients. II. Results of therapy with streptozotocin in 52 patients. Ann. Intern. Med. 79:101-118, 1973.
11. Broder, L.E. and Tormey, D.C. Combination chemotherapy of breast cancer. Cancer Treatment Reviews. (In press)
12. Carter, S.K. and Broder, L.E. The cytostatic therapy of hormone secreting tumors of the gastrointestinal tract. In Seminars in Oncology. New York, Grune & Stratton. (In press)
13. Cohen, M.H. Enhancement of antitumor effect of BCNU by various psychotropic drugs and caffeine. J. Pharmacol. and Exper. Ther. (In press.)
14. Cohen, M.H. Enhancement of the antitumor effect of 1,3-bis(2-chloroethyl)-1-nitrosourea by chlorpromazine and caffeine. J.N.C.I. 51:1323-1326, 1973.
15. Cohen, M.H. Signs and symptoms of bronchogenic carcinoma. In Seminars in Oncology. New York, Grune & Stratton. (In press)
16. Creaven, P.J. and Allen, L.M. Inhibition of macromoleculab biosynthesis in cultured L1210 mouse leukemia cells by thalicarpine (NSC 68075). Cancer Res. 33:3112-3116, 1973.
17. Creaven, P.J. and Allen, L.M. Renal clearance of camptothecin sodium: Effect of urine volume. Cancer Chemother. Rep. 57:175-184, 1973.
18. Creaven, P.J., Allen, L.M., Alford, D.A. and Cohen, M.H. Clinical pharmacology of isophosphamide. Clin. Pharmacol. Ther. (In press)
19. Creaven, P.J., Allen, L.M. and Williams, C.P. The interaction of the antineoplastic drug thalicarpine with aniline hydroxylase and microsoma-cytochrome of rat liver. Xenobiotica. (In press)
20. Creaven, P.J., Cohen, M.H., Hansen, H.H., Selawry, O.S. and Taylor, S.G. Phase I clinical trial of a single dose and 2 weekly schedules of ICRF 159 (NSC 129943). Cancer Chemother. Rep. (In press)
21. Creaven, P.J., Hansen, H.H., Alford, D.A. and Allen, L.M. Methotrexate in liver and bile after intravenous dosage in man. Brit. J. Cancer. 28:589-591, 1973.
22. Creaven, P.J., Muggia, F.M. and Allen, L.M. Plasma camptothecin (NSC 100800) levels during a 5-day course of treatment. Relation to dose and toxicity. Cancer Chemother. Rep. 56:573-578, 1972.
23. Harchaut, Y., Cohen, M.H., Stephen, D.H., et al. EBV virus antibodies in Hodgkin's disease. Cancer. (In press)
24. Harris, C., Primack, A. and Cohen, M.H. Alpha-1 antitrypsin in bronchogenic carcinoma. Cancer. (In press)
25. Matthews, M.J. Keynote address on pathology of lung cancer. Cancer Chemother. Rep. 4(part 3):15-16, 1973.
26. Matthews, M.J. Morphologic classification of bronchogenic carcinoma. Cancer Chemother. Rep. 4(part 3):299-307, 1973.
27. Matthews, M.J. Morphology of lung cancer. In Seminars in Oncology. New York, Grune & Stratton. (In press)
28. Matthews, M.J. Problems in morphology and behavior of bronchopulmonary malignancies. In Advances in Lung Cancer. Paris, Karger. (In press)

29. Matthews, M.J., Kanhouwa, S., Pickren, J. and Robinette, D. Frequency of residual and metastatic tumor in patients undergoing curative surgical resection for lung cancer. Cancer Chemother. Rep. 4(part 3): 63-67, 1973.
30. Napoli, L.D., Hansen, H.H., Muggia, F.M. and Twigg, H.L. The incidence of osseous involvement in lung cancer, with special reference to the development of osteoblastic changes. Radiol. 108:17-21, 1973.
31. Ny, A., Shalhoub, R.J., O'Connell, J.M.B., Matthews, M.J. and Higgins, G.A. Intractable ascites during maintenance hemodialysis. Med. Ann. Dist. Col. 42:277-279, 1973.
32. Primack, A. The production of markers by bronchogenic carcinoma: A review. In Seminars in Oncology. New York, Grune & Stratton. (In press)
33. Primack, A., Dhru, D., Kiryabwire, J.W.M. and Vogel, C.L. Clinical trials of combination chemotherapy with BCNU and vincristine in the treatment of malignant melanoma in Uganda. Cancer. 31:337-341, 1973.
34. Primack, A., Vogel, C.L. and Barker, L.F. Immunological studies in Ugandan patients with hepatocellular carcinoma. Brit. Med. J. 1:16-19, 1973.
35. Selawry, O.S. Initial therapeutic trial new drugs in lung cancer. Cancer Chemother. Rep. 4:295-305, 1973.
36. Selawry, O.S. Introduction to the 1st International Workshop to Therapy of Lung Cancer. Cancer Chemother. Rep. 4:61-62, 1973.
37. Selawry, O.S. Monchemotherapy of bronchogenic carcinoma with special reference to cell type. Cancer Chemother. Rep. 4:283-294, 1973.
38. Selawry, O.S. The role of chemotherapy in the treatment of lung cancer. In Seminars in Oncology. New York, Grune & Stratton. (In press)
39. Smith, J.C., Hansen, H.H., Selawry, O.S., et al. Serum zinc concentration: An unreliable parameter for diagnosing bronchogenic carcinoma. J.N.C.I. 51:1379-1382, 1973.
40. Straus, M. Lung cancer: 1974. In Seminars in Oncology. New York, Grune & Stratton. (In press)
41. Straus, M. The growth characteristics of lung cancer and its application to treatment design. In Seminars in Oncology. New York, Grune & Stratton. (In press)
42. Straus, M.J., Choi, S.C. and Goldin, A. Increased life-span in AKR leukemic mice treated with prophylactic chemotherapy. Cancer Res. 33:1724-1728, 1973.
43. Straus, M.J. and Goldin, A. Effects of primary dose schedules in cytoxan treatment of mouse leukemia L1210. Proc. Soc. Exp. Med. Biol. 145:1132-1138, 1974.
44. Tejada, F., Bias, W.B., Santos, G.W. and Zieve, P.D. Immunologic response of patients with acute leukemia to platelet transfusions. Blood. 42:405-412, 1973.
45. Vogel, C.L., Primack, A., Owen, R. and Kyalwazi, S.K. Effective treatment of Karposi's sarcoma with 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide (NSC 45388). Cancer Chemother. Rep. 57:65-71, 1973.

Serial No. NCI-3403
1. Medicine Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Clinical Trials and Miscellaneous Clinical Investigations

Previous Serial Number: Same

Principal Investigators: Vincent T. DeVita, Jr., George P. Canellos, Robert C. Young, Philip S. Schein, Bruce A. Chabner, Stephen H. Rosenoff, Susan P. Hubbard

Other Investigators: Ralph Johnson, Richard Sherins, First Year Clinical Associates, Medicine Branch, NCI

Cooperating Units: Radiation Branch, NCI, and Reproduction Research Branch, NICHHD.

Man Years:

Total:	10.6
Professional:	10.
Others	.06

Project Description:

The Medicine Branch is a major clinical facility of the National Cancer Institute. Its activities are divided between clinical therapeutic trials in patients with cancer, most of which are reported here under project number 3403, and clinical research efforts aimed at understanding the natural history of the disease in question, the interaction of the drugs used to treat them with the host, and parallel phenomena associated with cancer, chemicals, and the host. These latter studies are reported in companion projects number 6500, Clinical Pharmacology; number 3420, Kinetics of Cellular Proliferation; number 3405, Hematology Studies; and number 3404, Immunological Aspects of Cancer.

The clinical studies are conducted by the 4.5 senior staff (includes the Branch Chief and one investigator, Dr. Chabner from the Laboratory of Chemical Pharmacology, spending 50 percent of his time with the Medicine Branch), one research nurse, and six first-year Clinical Associates.

The clinical resources of the Branch are 19 beds and three outpatient afternoon clinics a week. The volume of patients studied in the Branch and the variety of diseases treated have increased significantly in the past year.

Over one-third of all patients admitted to NCI beds are admitted to the 19 Medicine Branch beds located on 12 West. Patients on Branch studies make over 6,000 outpatient visits per year and the total census now is approaching 600 patients. The trend to involvement in a greater variety of solid tumors continues.

A. Clinical Trials

1. Phase I studies of new antitumor agents or new approaches to the use of more established antitumor drugs.

2. Disease oriented studies:

    A. Hodgkin's disease
    B. Histiocytic lymphomas
    C. Lymphocytic lymphoma
    D. Burkitt's lymphoma
    E. Ovarian carcinoma
    F. Carcinoma of the breast
    G. Malignant melanoma
    H. Chronic myelocytic leukemia
    I. Osteogenic sarcoma
    J. Endocrine malignancies

In the latter half of this year new protocols are being developed for the study of carcinoma of the pancreas.

3. Achromycin, NSC 403169

Eighteen patients have received 100-675 mg/M^2 for an average of 30 days. The toxicity is primarily gastrointestinal with nausea and vomiting in 13 cases and mild thrombopenia in five cases. The antitumor responses were noted in one case of myeloma and soft tissue sarcoma.

4. MeCCNU

The Phase II trial of MeCCNU in disseminated malignant melanoma has been completed and is accepted for publication in Clinical Pharmacology and Experimental Therapeutics. Twenty-eight patients with disseminated malignant melanoma were treated with single doses of MeCCNU orally every six weeks. Six patients (31%) had a complete or partial regression of measurable disease. The median duration of the partial remissions was two months and for the one complete remission, 18 months. The median survival of those responding to therapy (10.6 months) was significantly longer than those who failed to respond (4.2 months). Regressions of skin, intracutaneous, and nodal disease were most common, but four patients had regressions of lung metastases and one patient had regression of bone metastases. Toxicity was primarily delayed thrombocytopenia and to a lesser extent leukopenia occurring within four to six weeks. The response rate, ease of administration, and absence of a sex difference in response rate make MeCCNU potentially useful in the chemotherapeutic management of disseminated malignant melanoma. MeCCNU is now being employed in randomized controlled trials comparing adjuvant chemotherapy and immunotherapy in patients with Stage II disease at high risk for disease recurrence after primary surgery.

5. Procarbazine

The Phase I trial of intravenous procarbazine has been completed and published. Seventeen patients in this study received doses ranging from 300 to 2,500 mg/M^2. Significant and disabling neurotoxicity requiring hospital care was observed at doses above 1,000 mg/M^2 and this is recommended as the highest dose which can be given on an outpatient basis. Liver dysfunction appears to be associated with increased drug toxicity.

6. Streptozotocin (V, 5XX, LA100)

Streptozotocin is a nitrosourea antitumor agent with the unique property of having activity against the L1210 system without myelosuppression. A Phase II study with streptozotocin has been conducted to determine whether this property can be exploited clinically.

Clinical antitumor activity has been demonstrated in patients with advanced refractory Hodgkin's disease and melanoma. No objective responses were observed in six patients with malignant carcinoid. The toxicologic properties of streptozotocin have been defined. Nausea and vomiting has been observed in all patients. Renal tubular toxicity is the major therapy limiting effect and can be anticipated by pretreatment measurements of urinary protein excretion. No myelosuppression has been observed. A return of bone marrow cellularity has been observed in patients on treatment who have severe myelosuppression prior to the initiation of treatment.

7. Hexamethylmelamine (HXM)

Previous Phase I studies have demonstrated HXM as a primary therapy has activity against ovarian and lung cancer without major bone marrow toxicity. Studies have been instituted to examine the antitumor activity of HXM in advanced alkylating agent resistant ovarian cancer to assess (1) the possible role of this agent in future combination treatment studies, (2) to determine whether cross-resistance exists between HXM and classical alkylating agents. Patients with ovarian cancer have been treated with initial doses of 8 mg/kg/day. Two of ten patients whose tumor was resistant to a classical alkylating agent have obtained partial responses of 3-4 months. The principal clinical toxicities observed have been nausea, mild myelosuppression, and in one patient, neurologic toxicity consisting of agitation and peripheral neuropathy which was reversible with dosage reduction. This study continues.

8. Adriamycin

Adriamycin is an anthracycline antibiotic with clinical antitumor activity against a wide range of human solid tumors. The compound has been used in cancer patients as a secondary therapy after failure on conventional treatment programs. Clinical activity has been demonstrated in patients with breast cancer, lymphoma, and sarcomas using a single intravenous dose of 60 mg/M^2. The principal toxicity has been depression of white blood cell count with a relative span of platelets.

B. Disease Oriented Programs

1. Ovarian Carcinoma

Ovarian carcinoma is the fourth most common killer of women among cancers and the incidence is increasing at the rate of 1% per year. The Medicine Branch became interested in the problem of ovarian cancer three years ago and extensively reviewed the subject in the New England Journal of Medicine in an article entitled, "The Treatment of Ovarian Carcinoma: Possibilities for Progress". We have begun a large scale study of the problem combining in-house, contract, and laboratory based investigations. Within the past year the Medicine Branch has completed a randomized prospective study comparing Melphalan with high dose intensive cyclophosphamide and a manuscript has been submitted for publication. Twenty-four fully staged, previously untreated patients with advanced ovarian carcinoma were prospectively randomized. Median duration of initial remissions (5 months and 6 months) and median duration of survival (15 months and 14 months) were similar for the two regimens but the toxicity of the intensive regimen was excessive. Followup indicates that long-term disease free survivals are possible in those patients who achieve complete remissions on chemotherapy alone, as three of the four patients achieving complete remission in the present study remain free of disease with a median survival in excess of 30 months. High dose intensive alkylating agent therapy in the manner used in the present study fails to enhance the response to chemotherapy and produces unacceptable toxicity.

As a consequence, the first trial we have initiated a second randomized prospective clinical trial comparing conventional Melphalan with a 4-drug combination known as Hexa-CAF composed of Hexamethylmelamine, Cyclophosphamide, Methotrexate and 5-FU.

The new Hexa-CAF regimen is as follows:

	Day 1	8	14	28
5-FU	600 mg/M^2	600 mg/M^2		
MTX	40 mg/M^2	40 mg/M^2		
CTX	150 mg/M^2 ⟶	⟶	⟶	
Hexamethylmelamine	150 mg/M^2 ⟶	⟶	⟶	

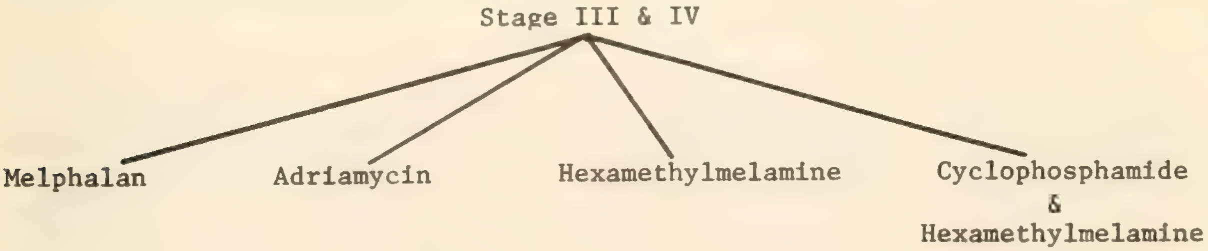
This regimen is repeated at monthly intervals for 6 cycles.

Other in-house studies have included, (a) initiation of a phase II effort with hexamethylmelamine, high dose procarbazine, and adriamycin in patients with refractory ovarian carcinoma; (b) kinetic studies on patients with ovarian ascites tumor; (c) the use of peritoneoscopy in the staging of ovarian carcinoma; (d) the use of lymphangiography in the staging and followup of patients with ovarian carcinoma.

In collaboration with the Radiation Branch of the National Cancer Institute, the program has been expanded to include patients with all stages of ovarian carcinoma. Patients with true Stage I will be randomized to receive no additional therapy or 12 months of intermittent Melphalan. Patients with Stage II disease will randomize to receive either radiotherapy alone or radiotherapy plus chemotherapy.

The Medicine Branch of the National Cancer Institute has embarked on a contractual arrangement with M. D. Anderson Hospital to identify antitumor activity of new and older drugs which might be useful in the treatment of ovarian carcinoma. The initial trial randomly allocates patients with advanced disease to either Melphalan, Hexamethylmelamine, or 5-FU. A crossover study will be employed to test for cross-resistance. Patients who fail on such regimens will then be treated with Phase I agents, the first of which will be MeCCNU.

To date 89 patients have been entered on trial. Preliminary results indicate a response rate of between 30.5-50.5% for Melphalan; 46.6-66% for Hexamethylmelamine, and 18.8-36% for 5-FU. The outstanding responses to Hexamethylmelamine are continuing to be studied and new trials will employ a cross over study to test cross resistance to conventional alkylating agents. In addition, Adriamycin will be tried primarily in advanced previously untreated disease and will replace 5-FU in the subsequent randomized trial. In addition, the two drug combination of Cyclophosphamide-Hexamethylmelamine will be compared to the other three single agents as follows.



Additional trials are planned to find other drugs that are effective in patients with ovarian cancer among active agents which have not received sufficient trial in ovarian cancer. These drugs include intravenous Procarbazine, Cytembena, high dose progesterones, Porfiromycin, and Streptozotocin.

Another contract program supervised by the Medicine Branch is underway at the Mt. Sinai School of Medicine in New York and is prospectively testing Methotrexate as a single agent compared with Melphalan. Information from these two trials should allow rational design of potentially useful combination chemotherapy in this disease.

As a result of the large number of new patients with ovarian carcinoma entering the Clinical Center, we have adopted the technique of peritoneoscopy in the staging of ovarian cancer. The results have been presented at the American Association for Cancer Research Meetings in Houston, April, 1974.

A major problem in managing patients with ovarian carcinoma is determining initial extent of disease (staging) and assessing residual tumor after therapy. Laparotomy often underestimates the extent of tumor, and the ability to repeatedly assess residual cancer by laparotomy is limited. Thirty patients referred to the NCI have had peritoneoscopy as part of their pre- and post-therapy evaluation. Thirty percent (9/30) of the patients had their stage changed by peritoneoscopy. Surgically undetected metastases to the right hemidiaphragm or liver capsule were found in 4/7 patients Stage I or II and in 13/18 patients Stage III at laparotomy. Twenty of thirty of the entire group had diaphragmatic implants. Peritoneoscopy added findings of value for followup in 27/30 patients. Sixty percent of these 27 patients had had no followable disease on physical exam; and in 37%, peritoneoscopy supplied the only followable findings. Seven "second look" peritoneoscopies were performed after apparently successful therapy. Four of seven patients were found to have residual intra-abdominal tumor. Peritoneoscopy has been demonstrated to be useful in staging and followup of patients with ovarian cancer.

2. Hodgkin's Disease (VI 6X1 LA100)

Most of the studies with Hodgkin's disease have been completed and a new protocol for Stage IV disease initiated. The first patients on study now have reached their tenth anniversary. Over 200 patients with Hodgkin's disease have been treated with the MOPP program. The general conclusions are as follows:

A. The complete remission rate in advanced disease remains 75%.

B. The median duration of complete remission is between 36 and 48 months.

C. No relapses have been noted after 42 months off therapy, an observation now confirmed at other institutions.

D. Maintenance therapy after complete remission has not proven useful in our Branch.

E. The median survival has still not been reached, but will definitely exceed nine years and has been projected by computer analysis of survival curves to be as long as 19 years.

F. The 5-year survival of complete responders with Stage III and IV disease is now 89%.

The current protocol will utilize radiation therapy to areas with known high risk of recurrence in conjunction with MOPP therapy in patients with advanced disease.

The study utilizing extensive x-ray therapy and MOPP chemotherapy in patients with Stage IIIB disease has been terminated. Although the survival of the 33 patients is good, the complication rate is high and probably not acceptable. These data are being analyzed for publication.

In 1967 a trial was instituted randomizing patients achieving complete remissions to either no therapy (control), intermittent BCNU therapy, or intermittent MOPP maintenance, the latter two therapies given at three monthly intervals for a total of 15 months.

This study has now been completed with a total of 90 patients entered with the following results and conclusions. The complete remission rate in this group (75%) is comparable to the original group (81%). Relapse rates in the three groups control (24%), MOPP (25%), and BCNU (16%), were not significantly different from one another, although those receiving no therapy relapsed sooner. Because these patients are easily retreated, there is no difference in survival at this point in time.

The five year survival of the complete responders in this study is 86% with a median duration of remission of 48 months. Using the worst slope of the survival curve, a projected median survival for the entire group of complete responders is 19 years. It should be emphasized that although this is a projected figure, many of the patients in the original study are approaching nine and ten years from the end of treatment. Using hard data, the median survival in this group of complete responders will be well in excess of seven years. In light of this observation, the above data seems like a reasonable estimate.

In 1969, patients with Stage IIIB disease only were assigned to therapy with six cycles of MOPP followed by total nodal irradiation.

Twenty-eight patients have been entered on study which is not yet completed. Preliminary conclusions are that (1) such an approach is complicated and difficult to complete successfully, and (2) the permanent morbidity may be high including an increased risk of developing second malignancies. In spite of these observations, the recurrence rate of Hodgkin's disease may be far less than our previous experience at NCI with Stage IIIB patients. Current plans are to combine MOPP plus x-ray to the dominant sites of relapse in patienss with stage IIIB and IV disease to attempt to prevent recurrences in high risk patients.

3. Lymphocytic and Histiocytic Lymphoma

a. Chemotherapy (VI, 6X1, LA100)

The results of CVP and MOPP combination chemotherapy programs in the treatment of 80 patients with advanced non-Hodgkin's lymphoma have been correlated with the Rappaport classification system. Thirty-six patients (45%) achieved a complete remission. Twenty-eight percent of all patients and 57% of complete remitters remain free of disease one to seven years with a projected median duration of remission in this group of three and a half years.

Significant differences in prognosis were found between histologic groups. Well-differentiated and nodular histology were positive determinants for response and survival. One-third of patients with advanced diffuse histiocytic lymphomas achieved complete responses and none have relapsed. Failure to achieve a complete response was associated with early demise.

b. Randomized Trial Between Radiotherapy and Chemotherapy in the Non-Hodgkin's Lymphomas (VI, 6X1, LA100).

Favorable therapeutic results for patients with advanced non-Hodgkin's lymphomas have been reported using total nodal and total body x-irradiation from the NCI's Radiation Branch. Because patients with apparently equivalently advanced disease were also doing well with combined chemotherapy, a controlled trial was established between the Medicine and Radiation Branches to determine the relative efficiency of each therapy by stage and histology subtypes.

After an extensive staging evaluation patients are randomized by stage, and histology to various treatment programs as follows:

1. Histiocytic and Mixed lymphomas

Stage I, II -- Local radiotherapy or local radiotherapy followed by C-MOPP
Stage III -- Total nodal radiotherapy or C-MOPP
Stage IV -- BACOP therapy (see below)

2. Lymphocytic lymphomas

Stages I and II -- Local radiotherapy only
Stage III -- Total nodal plus total body irradiation versus cytoxan, vincristine, and prednisone (CVP therapy)
Stage IV (also randomized by site of organ involvement) -- Total body irradiation plus irradiation to dominant disease versus CVP therapy.

To date, 100 Stage III, IV, lymphocytic, mixed lymphoma patients have been randomized. The data on the first 71 cases of lymphocytic lymphoma reveal an overall 86% response rate with 55% complete remissions divided equally between chemotherapy and TBI. The 48 month survival exceeds 80% in both groups. Nodular lymphomas had a higher overall response rate than diffuse lymphomas. Only 10 patients have died to date.

c. Evaluation of New Drug Combination (BACOP) in Advanced Histiocytic Lymphoma (VI, 6X1, LA100)

Evaluation of a combination of bleomycin, adriamycin, cyclophosphamide, prednisone, and vincristine (BACOP) in advanced histiocytic lymphoma. Despite the ability of some patients with Stage IV diffuse histiocytic lymphoma to achieve a complete remission and long survival, the overall median survival is 6 months after initiation of chemotherapy since less than half of patients can be rendered free of disease. Patients have demonstrated a deceivingly favorable initial response to treatment which is followed by a pattern of rapid relapse during recovery phases of MOPP chemotherapy cycles.

The BACOP protocol takes into account these clinical observations and represents an attempt to retard the regrowth of disease prior to the initiation of the next cytotoxic portion of the regimen.

A total of 27 patients have received this regimen, 10 of whom had been previously treated with either chemotherapy or radiotherapy. Six patients have achieved a complete remission, determined histologically one month after completion of therapy. Eleven patients obtained a partial response, ten are still on treatment with evidence of response.

Significant pulmonary toxicity has been encountered at total bleomycin doses less than 100 mg.

4. The American Burkitt's Lymphoma

Thirty cases of American Burkitt's lymphoma have been treated at NCI. Twenty-two received high dose 40 mg/kgm I.V. cyclophosphamide with 13 complete responses (59%). Of these four, all have relapsed within 12 months and three have died. The remainder are alive, free of disease, off treatment for up to 90+ months of followup. Factors which predict for complete remission and disease-free survival are: Stage I-II, or III with small tumor mass, an LDH <700 I.V./ml. and no relapse within 12 months, Eight of nine cases with presenting LDH <700 I.V./ml. are alive and free of disease and only one of thirteen with levels >700 I.V./ml are alive.

Pretreatment sera from 43 patients with African Burkitt's lymphoma were analyzed for the possible prognostic value of LDH, uric acid, lactate BUN, SGOT, and alkaline phosphatase.

Serum LDH levels were found to correlate with stage of disease, and appear to reflect tumor burden. Serum LDH level proved superior to all other chemical parameters in predicting risk of early relapse; patients with an LDH >275 I.U. had a significantly higher risk of early relapse, regardless of stage ($P < 0.01$). Fractionation identified LDH_2 and LDH_3 as the elevated isoenzymes.

In Burkitt's lymphoma patients with a large tumor burden, as indicated by a high LDH, rapid tumor lysis with chemotherapy may be associated with severe hyperphosphatemia, hypocalcemia, and hyperkalemia. These life-threatening metabolic abnormalities require that provision for renal dialysis be available prior to initiation of chemotherapy in such patients. LDH determinations should be included in future staging systems of Burkitt's lymphoma.

The wide variety of drugs used in these cases made analysis of survival on the basis of therapy impossible, but the treatment given to long-term survivors, noted in Table VI, does not reveal any specifically effective regimen. Although 62% of the patients failed to exceed a one year survival, the sensitivity of the tumor to therapy was indicated by the fact that 75% of the 36 patients on whom information on drug response was obtained were reported to have a more than 50% reduction in tumor size with the first course of therapy.

5. Combination Drug Therapy of Advanced Breast Carcinoma (VI, 6X1, LA100)

The FACP regimen (5-FU, MTX, cytoxan, and prednisone) has been applied to 33 women with advanced breast cancer (previously untreated with cytotoxic drugs). Twenty-two objective responses were seen including seven complete responses. The median duration of survival for responders will be 16-20 months with four months for non-responders. This regimen with modification (CMF) is being extensively evaluated by other groups. The toxicity is primarily hematopoietic.

6. Osteogenic sarcoma adjuvant study (VII, 7XX, LA100)

This attempt to improve the survival of patients with osteogenic sarcoma who are free of residual disease but with a high risk of relapse, a randomized adriamycin vs. no treatment trial has been initiated. Accrual has been slow and no meaningful data are currently available.

7. Streptozotocin in the Treatment of Malignant Insulinoma (V, 5XX, LA100)

Six patients with advanced malignant insulinoma have been treated with intravenous streptozotocin using a single weekly dose or daily treatment for five consecutive days. One patient has had an objective response which lasted for one year. There were no distinguishing features of this case that would predict for response other than the relatively small amount of hepatic metastases. The remaining patients have not had useful therapeutic responses.

C. Clinical Investigations

1. Staging in Lymphomas

The results of a comprehensive staging study of 100 patients with non-Hodgkin's lymphoma have been compiled and evaluated, indicating the widespread nature of this group of diseases at presentation and the ease with which Stage IV disease can be established in most patients without staging laparotomy. In our series 74% of patients had Stage IV disease at completion of staging and an additional 15% had nodal disease outside the usual radiotherapy fields, indicating the futility in using curative nodal radiotherapy in most patients. Liver involvement was found in 41% of patients, including 33 of 59 or 55% undergoing a complete liver evaluation (peritoneoscopy and laparotomy if required) and represented the only site of Stage IV disease in 19%. Staging laparotomy was required to establish or rule out Stage IV disease in only 32 of the 100 patients, and was useful in only eight patients, all of whom had liver involvement. Laparotomy was positive in none of seven patients with histiocytic lymphoma.

The study concluded that staging laparotomy was not warranted in patients with histiocytic lymphoma because of the infrequency of positive findings and the need to use systemic therapy in these patients regardless of the findings at laparotomy. In patients with mixed and lymphocytic lymphoma, laparotomy is not needed to diagnose Stage IV in the majority of patients, since a high field is obtained by closed methods of biopsy.

The results of this study will be incorporated into new protocols to be adopted for next year's studies of patients with lymphocytic and histiocytic lymphoma.

2. Peritoneoscopy

A. Staging of Lymphoma

As part of a staging program in non-Hodgkin's lymphoma, 77 patients were studied. Sixteen of 77 (21%) were positive for liver involvement by percutaneous biopsy. Forty-nine of 61 percutaneous biopsy negative patients then underwent peritoneoscopy with 18% positive. Of the 32 patients who went to subsequent laparotomy, eight (25%) were positive for liver involvement.

B. Staging of Ovarian Cancer

Nine of 30 consecutive patients with ovarian cancer had their stage changed as a result of the procedure. Twenty of 30 cases had diaphragmatic implants. In four of seven "second look" procedures residual tumor was found in the presence of complete clinical remission.

3. Study of Reproductive Capacity in Men and Women after Successful Intensive Combination Chemotherapy (V1, 6X1, SA100)

We have shown that intensive drug therapy may sterilize as many as 75% of men treated with MOPP or other intensive treatment for long periods of time. Recovery has been noted in some men.

We have initiated a similar study of the women in our studies.

Reproductive function is being evaluated in 19 women who have been treated with MOPP, MOMP, CVP, or cyclophosphamide for lymphoma, who are in remission and had received no therapy for at least six months. Initial evaluation by questionnaire and personal interview revealed abnormalities in menstrual function in two of three women. These patients are presently being evaluated endocrinologically with measurements of hypothalamic, pituitary, and ovarian hormonal function. It is anticipated that a small number of women will require ovarian biopsy at the conclusion of the evaluation to determine the etiology of the ammenorrhea. Approximately 1/3 of the women have delivered normal full-term fetuses without any apparent abnormalities detected. This study will be completed in the coming year.

4. Bone Marrow Involvement in Hodgkin's Disease and the non-Hodgkin's Lymphoma

In the past year our review of the bone marrow biopsies of 270 patients with Hodgkin's disease was completed. In 170 evaluable biopsies, tumor involvement was identified in 19 patients. Eighteen of these were treated with MOPP therapy and 11 achieved complete remission. The median duration of remission was equal to that of patients with other sites of involvement. Myelofibrosis or reticulum fibrosis was found in all patients with marrow involvement, and was reversible in patients achieving remission.

5. Second Malignancies in Hodgkin's Disease

Comparative studies of second tumors in long-term remitters of Hodgkin's disease revealed a high incidence of second tumors in patients who received intensive radiotherapy, relapsed and then received MOPP x 6. Of the 27 cases in that group, five (18.5%) second tumors (four solid tumors, one AML) have been seen. Fifteen have died (four from second tumor and 11 of recurrent Hodgkin's disease). Twleve are alive and free of Hodgkin's disease but one has a second tumor. If one includes all cases of combined intensive therapy, i.e. 27 ST-18 cases plus one, then the incidence in six tumors/56 cases (10.7%) with a median of 36+ months of followup as opposed to no greater than 3% with both modalities used alone.

Honors and Awards:

De. DeVita

1. "Hodgkin's Disease: A Decade of Progress", presented at the First Annual Clowes Memorial Lecture, Roswell Park Memorial Institute, Buffalo, N. Y., October, 1973.
2. Elected Member, Board of Directors, American Society of Clinical Oncology, 1973-1976.
3. Member, Nominating Committee, American Association for Cancer Research, 1974.
4. "Management of Hodgkin's Disease", presented at the American College of Physicians Regional Meeting, Rockefeller University, New York, March, 1973.
5. "The Non-Hodgkin's Lymphomas", presented at Grand Rounds, New Jersey College of Medicine.
6. Medical Ground Rounds, Memorial Hospital for Cancer and Allied Diseases, July 6, 1973.
7. "Chemotherapy of the Lymphomas", presented at Medical Grand Rounds, Chicago Medical School, September 15, 1973.
8. "The Management of Lymphomas", presented at the American College of Physicians Regional Meeting, September 19, 1973.
9. "Modern Approaches to Management of Cancer: Staging and Treatment of the Lymphomas", presented at Jacksonville, Florida, October 13 & 14, 1973.
10. "Chemotherapy of the Non-Hodgkin's Lymphoma", presented at the American College of Physicians Regional Meeting, November 3, 1973.

11. "Panel on Lymphomas", presented to the Washington, D.C. Medical Society, November 7, 1973.

12. "Panel on Management of Lymphomas", presented at the New York University Hospital, November 13, 1973.

13. "Hodgkin's Disease", presented at the Annual Cancer Seminar of the San Diego Medical Society, December 4, 1974.

14. "The Chemotherapy and Staging of Lymphomas", presented at the Cancer Seminar, Wesson Memorial Hospital, December 8, 1973.

15. "The Management of Lymphoma", presented at Medical Grand Rounds, North Shore Hospital, November 15, 1973.

16. "The Staging Chemotherapy of Lymphomas", presented at Cancer Seminar, Arnett Clinic, February 2, 1974.

17. "The Treatment of Lymphomas", presented at Medical Grand Rounds, Nassau County Medical Center, February 21, 1974.

18. Oncology Teaching Rounds, George Washington University School of Medicine, Washington, D.C., February 20, 1974.

19. "Chemotherapy of Genitourinary and Gynecologic Cancers", presented at Modern Concepts in Oncology, U.C.L.A. School of Medicine, March 3, 1974.

20. "Advances in the Chemotherapy of Cancer", presented at the William Beaumont Hospital, March 11, 1974.

21. "Hodgkin's Disease", presented at Duke University Medical School, Grand Rounds, March 22, 1974.

22. "New Approaches to Ovarian Carcinoma", presented at American Cancer Society Science Writers Seminar, March 22, 1974.

23. "Hodgkin's Disease", presented at Chemotherapy of Cancer Lecture Series, George Washington University School of Medicine, March 26, 1974.

24. Seminar on Chemotherapy of Breast and Ovarian Cancer, Allegheny General Hospital, Pittsburgh, Pa., April 11, 1974.

25. "Seminar on the Curability of Lymphomas", presented at the National Blood Club Meeting, Atlantic City, N.J., May 4, 1974.

26. "Chemotherapy of Cancer", presented at Sacramento Medical Society, Sacramento, Calif., May 21, 1974.

27. "Treatment of Hodgkin's Disease", presented at American Osteopathic Society Meeting, Washington, D.C., June 12, 1974.

Susan P. Hubbard, R.N.

1. Member, Task Force on the Future of Nursing Practice, Clinical Center, National Cancer Institute, 1973-1974.
2. Chairman, Recommendation Subcommittee, Task Force on the Future of Nursing Practice, 1974.
3. Member, Transfusion Committee, Medical Board, NIH
4. "The Role of the Research Nurse in Cancer Chemotherapy", presented to new orientees and senior nursing students, summer work program, NIH, June, 1973.
5. "The Role of the Cancer Chemotherapy Nurse in Cancer Research", presented to the Nursing Staff, Clinical Center, NIH, December, 1973.
6. "The Treatment of Cancer with Chemotherapy, Principles, and the Role of the Nurse", presented to senior class, Nursing School, Montgomery College, March, 1974.
7. "Cancer Nursing and the Therapy of Hodgkin's Disease and the non-Hodgkin's Lymphomas", presented at Nursing Symposium sponsored by the American Cancer Society, April, 1974.

Dr. Chabner

1. "Studies on the Pharmacology of Ara-C", presented at Roswell Park Memorial Institute, August 7, 1973.
2. "Staging of Non-Hodgkin's Lymphoma", presented at the International Conference on Non-Hodgkin's Lymphoma, London, England, October 10, 1973.
3. "Responsiveness and Toxicity in Cancer Chemotherapy", presented at Philadelphia General Hospital, March 12, 1974.
4. "Responsiveness and Toxicity", presented at Kingston, Ontario, March 13,
5. "Studies on Carbocypeptidase G.", presented at Yale University, April 5, 1974.
6. "Staging of Non-Hodgkin's Lymphoma", presented at A.S.C.O. Meeting, Houston, Texas, March 27, 1974.
7. "Clinical Studies of Carboxypeptidase G.", presented at the American Federation for Clinical Research Meeting, May 5, 1974.

8. "Activation and Degradation of Cytosine Arabinoside by Human Leukemic Cells", presented at U.C.L.A., June 7, 1974.

9. "Threshold Plasma Concentration of Methotrexate for Inhibition of DNA Synthesis *in vivo*", presented at the NCI, DCT Contractors Meeting, Hunt Valley, Md., November 20, 1973.

Dr. Young

1. Panel on Ovarian Cancer, A.S.C.O., March, 1974.

2. "Peritoneoscopy: A Valuable Tool for the Initial Staging and 'Second Look' in Ovarian Carcinoma", presented at the A.S.C.O. Meeting, Houston, April, 1974.

3. "Treatment of Hodgkin's Disease", presented at University of Miami, December, 1973.

4. "The Design of Clinical Trials in Ovarian Carcinoma", presented at the Society of Gynecological Oncologists, Key Biscayne, Fla, December, 1973.

5. "The Treatment of Ovarian Carcinoma", presented at the Baltimore Cancer Research Center, Baltimore, Md., December, 1973.

6. "The Treatment of Advanced Breast Cancer", presented at Winchester Hospital, January, 1974.

7. "New Advances in the Staging and Treatment of the Lymphomas", presented at St. Michael's Hospital, Newark, N.J., March, 1974.

8. "The Management of the Lymphomas", presented at Grand Rounds, New Jersey Medical Center, Newark, N.J., March, 1974.

9. "The Treatment of Ovarian Cancer: When to Use What and Why", presented at the Workshop of the American Society of Clinical Oncology, March, 1974.

10. "Treatment of Breast and Ovarian Cancer", presented at the Riverside Methodist Hospital, Columbus, Ohio, September, 1973.

11. "Treatment of Lymphomas", presented at the Education Committee Panel, American Society of Hematology Meeting, Chicago, Ill, December, 1973.

12. "Chemotherapy of Ovarian Cancer", presented at Suburban Hospital, Bethesda, Maryland, November, 1973.

13. "Chemotherapy of the Lymphomas", presented at Ohio State University, October, 1973.

14. "Treatment of Hodgkin's Disease and the Lymphomas", presented at Grand Rounds, Hershey Medical Center, Hershey, Pa., September, 1973.

Dr. Schein

1. "Current Concepts in Non-Hodgkin's Lymphoma", presented at the Children's Cancer Research Foundation, Boston, Mass., September 11, 1973.

2. "Results of Combination Chemotherapy for Non-Hodgkin's Lymphoma", presented at the Symposium on Non-Hodgkin's Lymphoma, London, England, October 12, 1973.

3. "The Non-Hodgkin's Lymphomas", presented at the Medical University of South Carolina, November, 1973.

4. "Lymphomas", presented at the American Society of Hematology Education Program, December 2, 1974.

5. "Current Concepts in Non-Hodgkin's Lymphomas", presented at Walter Reed Medical Center, Washington, D.C., December 13, 1973.

6. "Non-Hodgkin's Lymphoma", presented at the Memorial Sloan-Kettering Cancer Center, January 23, 1974.

7. "Lymphoproliferative Disorders and Chronic Lymphocytic Leukemia" presented at Georgetown University School of Medicine, Washington, D.C., February 6, 1974.

8. American Men and Women in Science-1974, Who's Who in the East, 1974.

9. Chairman, Gastrointestinal Tumor Study Group.

Dr. Canellos

1. "Androgen on Granulopoiesis", presented at the A.F.C.R. Meeting, Atlantic City, N.J., 1973.

2. "Breast Cancer, Non-Hodgkin's Lymphoma", presented at the International Congress of Chemotherapy, Athens, Greece, September, 1973.

3. "Second Tumors in Hodgkin's Disease", presented at the E.O.R.T.C. Meeting, Paris, France, July, 1973.

4. "Carcinogenesis by Anti-Tumor Therapy", presented at St. Bartholomew's Hospital, London, England, July, 1973.

5. "Breast Cancer", Second Year Lecture, George Washington University, May, 1973.

6. "Non-Hodgkin's Lymphoma", presented at the Washington Hospital Center, November, 1973.

7. "Myeloma", presented at the D.C. General Hospital, December, 1973.
8. "CVP Therapy vs. TBI", presented at the Non-Hodgkin's Lymphoma Conference, London, England, October, 1973.
9. "Myeloproliferative Diseases", Hematology Course, Georgetown University, February, 1974.
10. "Hodgkin's Disease", presented at the Delaware Cancer Society, February, 1974.
11. "Acute Leukemia Complicating Hodgkin's Disease", Educational Panel--Lymphoma, American Society of Hematology, Chicago, Ill, December, 1973.
12. "Lymphomas", presented at the American Cancer Society D.C. Medical Society Symposium, November, 1973.
13. "Nursing Symposium", presented at the American Cancer Society Meeting, April, 1974.
14. Visiting Professor of Medicine (Oncology), University of West Virginia, March, 1974.
15. Cancer Symposium, Northwestern University, January, 1974.
16. "Breast Cancer, Non-Hodgkin's Lymphoma", E.C.O.G. Breast Cancer Trial, A.A.C.R.-A.S.C.O., March, 1974.
17. "Chemotherapy of Hematologic Cancer", presented at the Department of Pharmacology, George Washington University, March, 1974.
18. American College of Physicians Course, Albany, N. Y., June, 1974.
19. "Lymphoma", Postgraduate Course, Harvard Medical School, May, 1974.
20. "Chronic Leukemia", presented at Walter Reed Hospital, April, 1974.

Publications:

Dr. DeVita

1. Graff, K.F., Simons, R.M., Yankee, R.F., DeVita, V.T., and Rogentine, D.G.: HLA Antigens on Hodgkin's Disease. J. Nat. Cancer Inst. (in press).
2. Johnson, R.E., DeVita, V.T., Kun, L.E., Chabner, B.A., Chretien, P.B., Berard, C.W., and Johnson, S.K.: Patterns of Involvement with Malignant Lymphoma and Implications for Treatment Decision Making. Brit. J. Cancer (in press).

3. Schein, P.S., O'Connel, M., Blum, J., Hubbard, S., McGrath, I., Bergevin, P., Ziegler, J., Wiernik, P., DeVita, V.T.: Clinical Antitumor Activity and Toxicity of Streptozotocin. Cancer (in press).

4. Johnson, R.E., Chretien, P.B., O'Conner, G.T., DeVita, V.T., and Thomas, L.B.: Radiotherapeutic Implications of Prospective Staging in One Hundred Consecutive Cases of Non-Hodgkin's Lymphoma. Radiology (in press).

5. Young, R.C. and DeVita, V.T.: Chemotherapy of ovarian cancer. Oncology Reviews (in press).

6. DeVita, V.T., Young, R.C., and Canellos, G.P.: Combination versus single agent chemotherapy. Proceedings of the Symposium on Combined Modalities in Cancer Therapy (in press).

Dr. Chabner

1. Chabner, B.A., et. al.: High-dose intermittent intravenous infusions of procarbazine. Cancer Chemo. Rep. 57: 361, 1973.

2. Chabner, B.A., et. al.: Percutaneous liver biopsy, peritoneoscopy, and laparotomy. An Assessment of relative merit in the lymphomas. Brit. J. of Cancer (in press).

3. Myers, C.S., Chabner, B.A., DeVita, V.T., and Gralnick, H.S.: Bone marrow involvement in Hodgkin's Disease: Pathology and response to MOPP chemotherapy. Blood (in press).

Dr. Schein

1. Schein, P.S., Chabner, B.A., Canellos, G.P., Young, R.C., Berard, C., and DeVita, V.T.: Potential for prolonged disease free survival following combination chemotherapy of non-Hodgkin's lymphoma. Blood 43: 181-189, 1974.

2. Schein, P.S., Chabner, B.A., Canellos, G.P., Young, R.C., Berard, C., and DeVita, V.T.: Results of combination chemotherapy of non-Hodgkin's lymphoma. Brit. J. of Cancer (in press).

3. Edelson, R.L., Kirkpatrick, C.H., Shevack, E.M., Schein, P.S., Smith, R.W., Green, I., and Lutzner, M.: Preferential cutaneous infiltration by neoplastic thymus-derived lymphocytes: Morphologic and functional studies. Ann. Int. Med. (in press).

4. Brereton, H., Anderson, T., and Schein, P.S.: Hyperphosphatemia and hypocalcemia complicating treatment of Burkitt's lymphoma. Arch. Int. Med. (in press).

5. Schein, P.S.: The use of chemotherapy in advanced ovarian cancer. Geriatrics 28: 89-95, 1973.

6. Schein, P.S., Chabner, B.A., Canellos, G.P., Young, R.C., and DeVita, V.T.: Non-Hodgkin's lymphoma: Patterns of relapse from complete remission after combination chemotherapy. Cancer (in press).

7. Schein, P.S., and Anderson, T.: Current approaches to the chemotherapy of lymphomas. Proc. 1st International Conf. on Cellular Kinetics and Antineoplastic Chemotherapy (in press).

8. Edelson, R.L., Schein, P.S., and Lutzner, M.: Sezary Syndrome, In, Current Dermatologic Management (in press).

9. Schein, P.S.: Endocrine tumors, In, Guide to Therapeutic Oncology (in press).

Dr. Young

1. Young, R.C. and DeVita, V.T.: Ovarian carcinoma: Clinical trials, prognostic factors, and criteria for response. Prognostic Factors and Criteria of Response to Cancer Therapy, Ed. M. Staquet, Brussels, Belgium (in press).

2. Young, R.C., Hubbard, S. P., and DeVita, V.T.: The chemotherapy of ovarian cancer. Cancer Treatment Rev. (in press).

3. Young, R.C., Canellos, G.P., Chabner, B.A., Schein, P.S., and DeVita, V.T.: Maintenance chemotherapy for advanced Hodgkin's disease in remission: A cautionary note. Lancet 1: 1359-1343, 1973.

4. Young, R.C., Walker, M.D., Canellos, G.P., Schein, P.S., Chabner, B.A., and DeVita, V.T.: Initial clinical trials with Methyl-CCNU 1-(2-Chloroethyl)-3-(4-Methyl Cyclohexyl)-1-Nitrosourea (MeCCNU). Cancer 31: 1164, 1973.

Dr. Canellos

1. Canellos, G.P., DeVita, V.T., Arseneau, J.C., and Johnston, R.C.: Carcinogenesis by cancer chemotherapeutic agents: Second malignancies complicating Hodgkin's disease in remission. Recent Results in Cancer Research, Springer-Verlag (in press).

2. Canellos, G.P., DeVita, V.T., Gold, G.L., Chabner, B.A., Schein, P.S., Young, R.C.: Cyclical combination chemotherapy in the treatment of advanced breast carcinoma. Brit. Med. J. 1: 218-220, 1974.

3. Canellos, G.P., DeVita, V.T., Young, R.C., Chabner, B.A., Schein, P.S., and Johnson, R.E.: Intensive chemotherapy for advanced lymphocytic lymphoma: Randomized comparison with intensive radiotherapy. Brit. J. Cancer (in press).

4. Arseneau, J.C., Canellos, G.P., Sherins, R., and DeVita, V.T.: Recently recognized complications of cancer chemotherapy. Ann. N. Y. Acad. Sci.(in press).

Serial No. NCI-3404
1. Medicine Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immunologic Aspects of Cancer

Previous Serial Number: Same

Principal Investigator: Robert C. Young, Vincent T. DeVita, Jr., Philip S. Schein, Bruce A. Chabner, Stephen H. Rosenoff

Other Investigators: John E. Bennett and Michael Blaese

Cooperating Units: Clinical Investigations Branch, NIAID, and Immunology Branch, NCI

Man Years:

Total:	0.2
Professional:	0.2
Other:	0

Project Description:

1. To study the immunologic status of patients with malignancies and relate these findings to progression or arrest of the disease and survival.

2. To exploit the host's immunologic system as an adjuvant to chemotherapy of tumors.

3. To exploit antigenic markers in the therapy of patients with cancer.

4. To identify, study, and develop therapy for infectious complications of patients with cancer.

A. Immunology and Cancer

Interest has centered around the role of Transfer Factor (VVI, 7 XX, EA100) in patients with Hodgkin's disease. Patients with Hodgkin's disease seem to have a unique defect in their inability to respond to transfer factor. Since the defect in delayed hypersensitivity seems to be relative rather than absolute, we have begun testing patients with massive doses of transfer factor to see if it is possible to confer delayed hypersensitivity in Hodgkin's disease patients prior to therapy. To date such transfer has not been achieved even with massive doses of transfer factor.

A comprehensive re-evaluation of the immune status of patients who enter remission is now underway and includes retesting patients for delayed hypersensitivity after they achieve complete remission. Thus far, approximately 60% of those patients anergic before therapy have recovered the ability to respond to a skin test antigen after therapy. Studies to see whether they can now respond to transfer factor are now underway. Evidence thus far accumulated would suggest that the immunologic defect present with Hodgkin's disease has been eliminated by successful chemotherapy.

1. Immunologic Studies in Non-Hodgkin's Lymphomas (VII, 7XX, SA25, EA75)

Use of serum and skin testing to define the immunological defect in non-Hodgkin's lymphoma is underway. Studies have demonstrated a correlation with lymphocyte depleted histology. A prospective study investigating skin test reactivity to stage B+T cell markers and response to therapy is being conducted in all histologic forms of non-Hodgkin's lymphoma.

2. Tumor Specific Antigen in Ovarian Carcinoma (V, 5XX, LA100)

Associated with the recent clinical interest in ovarian cancer, samples of human ascites tumor have been grown in cell culture, and a program of antigenic extraction and animal immunization is underway to produce high titer specific antibody.

3. HLA Antigens in Hodgkin's Disease (VII, 7XX, EA100)

The HLA antigen system is a major histo-compatibility locus in man. Over 25 different HLA antigens have been identified. Susceptibility to some leukemogenic viruses are clearly related to the alleles of the major histocompatibility locus in several animal species. An examination of HLA antigens in a series of patients with Hodgkin's disease has been completed.

The results suggest that disease susceptibility and subsequent histologic pattern may be influenced by factors related to the HLA pheno-type of the host. A more recent observation of the waxing and waning of the expressed HLA antigens in patients at varying time intervals is being explored.

B. Infections and Cancer

1. Pneumocystis Carinii (VI, 6X1, SA50, EA50)

Investigations into the identification and treatment of this illness and our published results have been summarized in the Annual Reports, 1969-1973. The current study program involves a randomized trial of known or presumed pneumocystis infections to either Pentamidine or Pyrimethamine and Sulfadiazine. Results thus far indicate that both regimens are effective in the disease but both have toxicities and problems associated with their administration. Data on the activity, toxicity, and the utility of these two regimens will be of considerable aid in managing this previously uniformly fatal infectious complication of malignancy.

2. HLA Antigens in Hodgkin's Disease (V11, 7XX, EA100)

The HLA antigens in 103 Caucasian Hodgkin's disease patients were compared with 459 healthy Caucasian controls. Only A5 were found to be significantly more frequent in the total patient population and this increase was related to histologic type. A5 was increased in the mixed cellularity and lymphocyte predominance histologic subtypes. It was not elevated in patients with the nodular sclerosis variety but seemed related to clinical stage of disease. A1, A8, and W18 were significantly increased in mixed cellularity but not in the other histologic types. Our findings suggest that the clinical and pathologic pattern of this disease may be influenced by the HLA phenotype of the host. Differences in the histologic subtypes in a patient population may account for variations in HLA antigen in various studies. This study has been completed.

Honors and Awards:

Dr. Young

1. "The Therapy of Pneumocystis Carinii Infections". Presented at Combined Clinical Staff Conference, March, 1974.

2. "Treatment of Pneumocystis Carinii". Presented at Symposium on Pneumocystis Carinii, Holiday Inn, Bethesda, Md., December, 1973.

Publications:

Dr. Young

1. Young, R.C. and DeVita, V.T.: The treatment of pneumocystis carinii pneumonia: Current status of the regimens of pentamidine isethionate and pyrimethamine-sulfadiazine. J.N.C.I. (in press).

2. DeVita, V.T., Goodell, B., Hubbard, S., Geelhoed, G.W., and Young, R.C.: Pneumocystis pneumonia in patients with cancer: Clinical setting. J.N.C.I. (in press).

3. DeVita, V.T. and Young, R.C.: Infection and cancer: Old friends. Ann. Int. Med. 79: 597-599, 1973.

Dr. DeVita

1. DeVita, V.T.: Lymphocyte reactivity in Hodgkin's disease: A lymphocyte civil war. New Eng. J. Med. (in press).

Serial No. NCI-3405
1. Medicine Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Hematologic Investigations

Previous Serial Number: Same

Principal Investigators: George P. Canellos, Vincent T. DeVita, Jr., Jacqueline Whang-Peng, Philip S. Schein, Bruce A. Chabner, Robert C. Young

Other Investigators: Tom Anderson and William Mead
Louis Gelrud, Gerald Johnston

Cooperating Units: Surgery Branch, NCI; Cytogenetics Section and Nuclear Medicine Dept., Clinical Center, NIH; Laboratory of Clinical Investigation, NIAID

Man Years:

Total:	1.6
Professional:	1.2
Other:	0.4

Project Description:

Clinical and laboratory investigations in patients with chronic myelogenous leukemia have been facilitated by the fact that patients with this disorder have (1) abundant granulocytes for periods of time off treatment, (2) undergoes a transition from a premalignant to a highly malignant disease as part of its natural history. In the past year, efforts have been primarily directed toward using the granulocyte of this disease as a human tissue source for the study of a new granulocyte-labelling isotope, ^{67}Gallium.

A. Cytogenetic studies (V 5XX, LA25, SA50, EA25)

Collaboration with Dr. J. Whang-Peng has continued in an attempt to define a correlation between cytogenetic findings during blastic transformation and response to specific therapy. This represents the most completely studied groups of patients from diagnosis through to blastic crisis. Some of the more significant correlations are included under the discussion of blastic crisis therapy.

1. Chronic Phase Chemotherapy (VI, 6X1, SA50, EA50)

Forty patients have been randomized between dibromomanniton (DBM) and busulfan in order to investigate the comparative effectiveness of the former drug, a relatively new agent, in a group of previously untreated, Philadelphia chromosome positive recently diagnosed patients. The study has been completed and has required 5 years to properly evaluate this new agent in CGL. There were 16/20 complete remissions with DBM and 14/20 with busulfan. Failures were due to early blastic crisis and severe hypoplasia in 2 cases treated with busulfan. Mean time to remission was shorter with DBM (72 days) vs. 100 days for Busulfan. Busulfan resulted in a more prolonged myelosuppressive effect such that 9/14 remitters required a median time of 9 months before drug could be readministered for maintenance compared with only 3/16 DBM patients who required a delay. Extensive histologic evaluation revealed no differences in the extent of cellular atypia produced in lung or bladder tissue. The survival for the groups did not significantly differ.

2. Splenectomy (VII, 7XX, EA100)

The role of splenectomy on the natural history of chronic granulocytic leukemia is being investigated. Thus far 22 patients have had a prophylactic splenectomy. Thirteen patients have entered blastic transformation and twelve of these received chemotherapy. The median survival of this latter group is 6 months (range 3-10), despite absence of complete remission. The general supportive care of such patients was felt to have been markedly improved. There was only one complication of the splenectomy--postoperative bleeding in a single case.

3. Treatment of Blastic Phase of Chronic Granulocytic Leukemia (VI, 6X1, LA 100)

Since the demonstration that vincristine and prednisone were capable of inducing hematologic remission in 30% of a series of 30 consecutive patients with blastic crisis of CGL, a randomized trial of this combination versus arabinosyl cytosine (ARA-C) (200 mg/m^2) and 6-thioguanine (200 mg/m^2) was begun. To date 25 patients have been studied. Ten have received ARA-C/6TG with one complete and 2 good partial responses. Fifteen have received vincristine, prednisone with one complete remitter in the latter group presented with hypodiploidy (44 Ph'). Including cases from our previous study 5/6 patients who presented with hypodiploidy at time of blastic crisis achieved a complete hematologic remission. We have a further patient treated in consultation at D.C. General Hospital who survived 36 months after complete remission with VCR/Pred., she was also hypodiploid. This makes a total of 6/7 cases who achieved remission only with VCR/Pred. Crossover to opposite drug regimen was attempted in 15 cases with a partial response in one case.

C. Laboratory Investigations

1. The radionuclide ^{67}Ga has been extensively investigated. In summary, studies to date, some of which have been published, have revealed (1) marked affinity of normal and leukemic granulocytes for the isotope. Further, this has been correlated with the presence of lysosomes. In addition, lymphocytes under the influence of PHA were shown to increase their capacity to bind ^{67}Ga which is a function of the appearance of lysosomes after PHA. Mouse melanoma cells have been studied by subcellular fractionation density gradients with marked localization of ^{67}Ga to melanosomes. Thus, we may have discovered a specific marker isotope for these organelles.

2. Studies of in vitro granulocyte production of patients with the lymphomas. (VI GX1 LA58 SA50)

   This study has been completed and prepared for publication. Colony formation in vitro was studied in patients with the various lymphomas before and after therapy.

   The major findings were that in vitro growth was related to the virulence of the disease as assessed by lymph node histology, that is, those patients with the more aggressive histologic types had the greater suppression of in vitro growth. This tended also to reflect volume of disease in Hodgkin's disease. The same correlation was noted in the non-Hodgkin's lymphoma. The nodular histology had normal in vitro growth, diffuse histology was associated with marrow suppression.

   Surprisingly, proven marrow invasion did not compromise in vitro growth over and above that related to the histology of the disease, e.g., nodular lymphomas with marrow involvement had normal in vitro growth. The data in general suggest a systemic effect of a tumor product on marrow growth which may relate to tumor volume or rapidity of growth.

3. Investigation of Usefulness of Nitroblue Tetrazolium (NBT) test in determining fewer source in determining fever source in patients with lymphomas (VI, 6X2, Ea100)

   A prospective evaluation of the nitroblue tetrazolium (NBT) dye reduction reaction has been completed and revealed that the response of neutrophils from 76 lymphoma patients was comparable to a control population of 44 patients without underlying malignancy. The NBT test proved to be a useful adjunct in distinguishing fever as a systemic manifestation of underlying lymphoma from that secondary to bacterial infection. In contrast to lymphoma patients with "B" fever, bacterially-infected lymphoma patients had elevated resting NBT scores comparable to a control population with bacterial infections. Lymphoma chemotherapy, including prednisone, did not inhibit neutrophilic response to infection or latex particle phagocytosis, but procarbazine may have produced a false positive response in some patients. It was found that it is essential to perform a simultaneous latex particle stimulation control to rule out an acquired intracellular defect that would otherwise be interpreted as a false negative response.

4. Studies of Leukocyte NADH Oxidase (VI, 6X2, SA50, Ea50)

Neutrophil NADH oxidase has been suggested as the primary enzyme involved in bacteriocidal activity, and deficient in patients with chronic granulomatous disease who are predisposed to infection. A sensitive micro assay system has been developed for the measurement of NADH oxidase in order to determine the effect of disease and chemotherapy on the enzyme's activity.

Honors and Awards

Dr. Canellos

1. "Myeloma". Presented at American Federation of Clinical Research, Atlantic City, N.J., May, 1973.
2. "Myeloma". Presented at D.C. General Hospital, Washington, D.C., Dec., 1973
3. "Chemotherapy of Leukemia". Presented at George Washington University, March, 1974.
4. "Myeloproliferative Disorders". Presented at Georgetown University, Feb., 1974.
5. "Dibromannitol in the Treatment of CGL". Presented at American Socity of Clinical Oncology, Atlantic City, N.J., April, 1973.

Publications:

Dr. Canellos

1. Canellos, G.P., DeVita, V.T., Young, R.C., Chabner, B.A., and Schein, P.S.: Current Therapeutic Aspects of Chronic Granulocytic Leukemia. Proc. of the 7th Natl. Cancer Conf., p. 351-357, 1973.
2. Canellos, G.P. and Whang-Peng, J.: The Philadelphia Chromosome Positive Preleukemic State. Lancet 2: 1227, 1972.
3. Rosenoff, S.H., Canellos, G.P., O'Connell, M., Wiernik, P.H.: Mediastinal Adenopathy in Granulocytic Leukemia. Arch. Int. Med. (in press)
4. Arseneau, J.C., Bagley, C., Anderson, T., Canellos, G.P.: Hyperkalemia: A Sequel to Chemotherapy of Burkitt's Lymphoma. Lancet 1:10, 1973.

5. Canellos, G.P.: Hypercalcemia in Lymphoma and Leukemia. Ann. N. Y. Acad. Sci. (in press).

6. Arseneau, J.A., Aamodt, R., Johnston, G.S., and Canellos, G.P.: Evidence For Granulocytic Incorporation of ^{67}Gallium in Chronic Granulocytic Leukemia. J. Lab. Clin. Med. 83: 489, 1974.

7. Gelrud, L.G., Arseneau, J.C., Milder, M.S., Kramer, R.J., Swarm, S.J., Canellos, G.P., and Johnston, G.S.: The Kinetics of Gallium-67 Incorporation Into Inflammatory Lesions: Experimental and Clinical Studies. J. Lab. Clin. Med. 83:496, 1974

8. Canellos, G.P.: Treatment of Chronic Granulocytic Leukemia. In Conn (ed): Current Therapy, 1974.

9. Anderson, T, Schein, P.S., Jencko, J.A., Binder, R.H.: The NBT Dye Test in Determining Fever Source in Lymphoma. Cancer (in press).

10. Mason, J.E., DeVita, V.T., and Canellos, G.P.: Thrombocytosis in Chronic Granulocytic Leukemia. Indidence and clinical significance. Blood, (in press).

Serial No. NCI-3420
1. Medicine Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Kinetics of Cellular Proliferation

Previous Serial Number: Same

Principal Investigators: Robert C. Young, Vincent T. DeVita, Jr., Philip S. Schein, Bruce A. Chabner, Stephen H. Rosenoff, and Harmar Brereton

Other Investigators: Charles E. Myers, Vincent T. Oliverio

Cooperating Units: Laboratory of Chemical Pharmacology, NCI
Radiation Branch, NCI

Man Years:

Total:	3.4
Professional:	1.6
Other:	1.8

Project Description:

The goals of the tumor cell kinetic program of the Medicine Branch over the past five years have been:

1. To examine and develop methodology that might be useful in obtaining cell kinetics information in individual patients in a time period that may allow application of these data to the patient's therapy.

2. To describe the kinetics of human tumors, compare them to animal models, and to compare results to normal tissues, to the same tumor in different patients, and to different metastatic sites of the same tumor in the same patients.

3. To examine the manner in which cell cycle characteristics are changed in response to exposure to drugs.

4. To approach the problem of manipulating tumor growth characteristics in such a way as to increase its susceptibility to chemotherapeutic agents.

A. Animal Studies

A series of studies have been completed on the effect of antitumor agents on DNA synthesis in normal and tumorous target tissues in vivo. Among the drugs studied are BCNU, Cytoxan, MeCCNU, ARA-C, Benzoate ARA-C, Cyclocytidine, Methotrexate, and Adriamycin. All these agents in sensitive tumor systems show a differential sensitivity of the tumor compared to the normal tissues. Proper sequences of drug therapy can be developed by taking into consideration these kinetic differences. Studies have been expanded to include investigations not only in ascites tumors but now in solid tumors such as B16 Melanoma and Lewis Lung Carcinoma.

B. Kinetic-Pharmacokinetic Studies

In cooperation with the Laboratory of Chemical Pharmacology, NCI, simultaneous studies on the pharmacokinetics of drugs such as Methotrexate and 5-FU, and the kinetic alterations in DNA synthesis produced by these agents in vivo have been studied. The results of such studies have been published and presented at national meetings and have defined the threshold methotrexate concentration needed to suppress DNA synthesis in normal and tumorous tissues in animals and man. Similarly with 5-FU we have defined the ratio of FdVMP/dVMP required for recovery of DNA synthesis in vivo after 5-FU.

C. Human Studies

Mtx studies on the effect of Mtx on the suppression and recovery of DNA synthesis in human bone marrow and human ovarian carcinoma ascites cells have been completed in three patients with a simultaneous study of blood and ascites levels of Mtx. As with animals, DNA synthesis in humans in vivo seems to recover when the concentration of Mtx in the blood falls to 1×10^{-8}M or lower and that the kinetic suppression and recovery can be predicted from a knowledge of the patterns blood levels of the agent. In these three patients, all of whom failed to respond to Mtx, the ovarian ascites tumor recovered at concentration of Mtx and one log higher than did the normal bone marrow.

When the B_{16}-Melanoma-MeCCNU model is completed, we plan to use a similar system in man with simultaneous studies on bone marrow and melanoma nodules, to see if schedules of MeCCNU can be derived by studying differential DNA dynthesis recovery patterns for the human marrow and the melanoma nodules.

D. Adriamycin-Kinetic Studies

Studies have been performed on the effect of Adriamycin on normal and tumorous tissues in animals. Alterations in DNA synthesis have been demonstrated in the heart of such animals and is temporally related to fatal cardiac toxicity as demonstrated histologically. Further studies on the nature of this alteration in cardiac muscle DNA synthesis are presently underway. This model may provide a useful system for screening derivatives of adriamycin which minimize cardiac toxicity.

Honors And Awards

Dr. Young

1. "Alterations of DNA synthesis in heart, bone marrow, and gastrointestinal mucosa induced by adriamycin in vivo: Relationship to fatal toxicity". Presented at American Society of Clinical Oncology Meeting, Houston, Texas, March, 1974
2. "Kinetic alterations induced by benzoate ARA-C and ARA-C in tumorous and normal tissues". Presented at, American Society of Clinical Oncology meeting, Houston, Texas, March, 1974.
3. "Kinetic alterations induced by Methl-CCNU in normal tissues and melanoma in vivo: Implications for chemotherapy". Presented at American Society of Clinical Oncology Meeting, Houston, Texas, March, 1974.
4. "Infectious Complications of Malignancy". Presented at Hershey Medical Center, Hershey, Pa. Sept.,1973.
5. "Kinetics of Tumor Growth". Presented at MIT Luncheon, Brookings Institute, Washington, D.C., Oct., 1973.
6. "Integration of Pharmacokinetics and Chemotherapy". Presented at National Cancer Advisory Council., Oct., 1973.
7. "Kinetic Alterations Induced by Chemotherapy ". Presented at Hunt Valley Contractors Meeting, Baltimore, Md. Nov., 1973.
8. "Repair of Normal Hematopoietic Tissue Following Chemotherapeutic Suppression: Comparative Analysis of Biochemical and Cell Culture Techniques". Presented at, American Society of Clinical Investigation, May, 1974
9. "A New Specific Stoichiometric Assay for 5-Fluorodeoxyuridylate: Use in Kinetic Studies of 5-Fluorouracil Effect in Normal and Tumorous Tissue. Presented at, American Society of Clinical Investigation, May, 1974.
10. "Cell Kinetics and the Chemotherapy of Cancer". Presented at, St. Joseph's Hospital, Orange, California. Dec. 4., 1973.

Publications:

Dr. Young

1. Rosenoff, S.H., Bostick, F., and Young, R.C.: Alterations in DNA Synthesis Induced by BCNU in Normal and Tumorous Tissues: Implications for Optimal Chemotherapy. Biochemical Pharmacology (in press).

2. Myers, C.E., Young, R.C., Johns, D.G., Chabner, B.A.: Enzymatic Assay of Intracellular 5-Fluorodeoxyuridine, 5-Monophosphate, and Deoxyuridine-5-Monophosphate Following 5-Fluorouracil. Cancer Res. (in press).

Serial No. NCI-6500
1. Medicine Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: "Clinical Pharmacology -- Mechanism of Action, Physiologic Disposition, Metabolism, and Toxicity of Anticancer Agents"

Previous Serial Number: Same

Principal Investigators: Philip Schein, Bruce Chabner, Tom Anderson, Robert Young, George Canellos, Vincent DeVita, Jr.

Other Investigators: David Johns, David Cooney, Ronald Kahn

Cooperating Units: Laboratory of Chemical Pharmacology and Toxicology, NCI
Diabetes Section, Endocrinology Branch, NIAMDD

Man Years:

Total:	2.9
Professional:	1.4
Other:	1.5

Project Description:

A significant area of interest in medical oncology is the identification and understanding of mechanisms of action of new anticancer agents. The Section of Clinical Pharmacology is involved in the design and conduct of new drug (Phase I) trials and development of methodology for analysis of new and old chemotherapeutic agents and conduct of studies of absorption, distribution, metabolism, and excretion of cancer chemotherapeutic agents used singly or in combination while they are employed clinically. Laboratory studies are aimed at determination of antitumor and toxic drug effects that may lead to development of new agents with a higher therapeutic index.

A. Nitrosourea Antitumor Agents

1. Structure-Activity Relationships for reduced bone marrow toxicity.

The treatment-limiting toxicity of the principle nitosourea anti-tumor agents in clinical use, BCNU (NSC-409962) and CCNU (NSC-79037), is myelosuppression;LD_{10} doses in mice produce a 60-75% reduction in both peripheral white blood cell count (WBC) and bone marrow DNA synthesis. Structure-activity studies have identified chemical features that may reduce this toxicity; the placement of an aminoglucose carrier on the N-3 position of 1-methyl-1-nitrosourea, a bone marrow toxin in animals, results in the formation of the bib-myelosuppressive but diabetogenic antitumor agent, streptozotocin (NSC-85998).

The pharmacologic properties of two newly synthesized aminoglucose chloroethyl nitrosourea agents have been examined. 3-(tetra-acetyl glucopyranose-2-yl)-1-(2-chloroethyl)1-nitrosourea, GCNU, (NSC-114460) at an LD_{10} dose of 15 mg/kg without accompanying leukopenia of inhibition of bone marrow DNA synthesis.

DCNU (NSC-178238) differs from DCNU by the absence of the acetates on the glucosamine carrier, and solubility in water; and LD_{10} dose, 15 mg/kg, resulted in a 701% and > 310% ILS in mice treated on day 2 and 6 of L-1210 tumor growth respectively, with 30-60% 90 day survivors. DCNU produced no significant depression in normal bone marrow DNA synthesis or peripheral WBC in contrast to a sustained 90% inhibition in L1210 DNA synthesis.

These structure-activity studies demonstrate that nitrosourea bone marrow toxicity can be modified without interfering with antitumor activity.

2. Mechanism of diabetogenic action of streptozotocin and related compounds.

Streptozotocin produces diabetes in laboratory animals and has demonstrated clinical antitumor activity against pancreatic islet cell carcinoma.

The diabetogenic activity of streptozotocin has been correlated with a reduction in pyridine nucleotide synthesis in the mouse pancreatic islet. To determine the specificity of this reaction the diabetogenicity, comparative studies of streptozotocin, its cytotoxic moiety 1-methyl-1-nitrosourea, and alloxan have been performed. Streptozotocin administered i.p. produced a dose-related reduction in islet NAD which was proportional to the degree of diabetogenicity. A diabetogenic dose, 200 mg/kg, attained a peak plasma N-nitroso intact streptozotocin concentration of 0.224 µmol/ml, and reduced the mean islet NAD from a control of 0.78 to 0.15 pmol. At borderline, 150mg/kg and non-diabetogenic, 100 mg/kg, doses, plasma concentrations reached 0.161 and 0.136 µmol/ml. and NAD was 0.36 and 0.86 pmol/islet respectively. 1-Methyl-1-nitrosourea, 100 mg/kg, attained a maximum N-nitroso intact 1-methyl-1-nitrosourea concentration of 0.162 µmol/ml and reduced the mean NAD to 0.58 pmol/islet, and was nondiabetogenic. 200 mg/kg attained a peak plasma concentration of 0.344 µmol/ml and depressed NAD to 0.38 pmol/islet, and was inconsistently diabetogenic. Islet NAD of 0.4 pmol/islet or greater is required for integrity of the beta cell. A diabetogenic dose if alloxan, 500 mg/kg, did not depress NAD, 0.85 pmol/islet, therefore confirming that its mechanism of diabetogenicity differs from that of streptozotocin. In vivo uptake of [^{14}C-methyl] streptozotocin by islets was 3.8 times that of [^{14}C-methyl] 1-methyl-1-nitrosourea, whereas uptake by the exocrine pancreas favored 1-methyl-1-nitrosourea over streptozotocin 2.4:1. The decreased islet uptake of 1-methyl-1-nitrosourea correlates with the3.5 times increased molar dosage required to produce islet NAD depression comparable to that of streptozotocin, 150 mg/kg. These studies indicate that the glucose carrier of streptozotocin facilitates uptake of its cytotoxic group, 1-methyl-1-nitrosourea, into islets.

2-Deoxy-2-(((ethylnitrosamino)carbonyl)amino)-D-glucopyranose (DGNU) is a newly synthesized glucose-containing nitrosourea differing in chemical structure from the diabetogenic agent streptozotocin by the presence of an ethyl end group on the nitrosourea moiety. When administered at a dose of 2500 mg/kg, DGNU was diabetogenic as confirmed by plasma glucose and insulin concentrations, and histologic evidence of pancreatic islet necrosis. With streptozotocin, this diabetogenic activity could be correlated with a reduction of mean pancreatic islet nicotinamide adenine dinucleotide (NAD) content, 0.11 pmol/islet as compared to a control mean of 0.83 pmol/islet by 3 hours after administration. The relatively large dose of drug required for pharmacologic activity is consistent with structure-activity studies demonstrating that nitrosamine and nitrosourea compounds possessing an ethyl end group require approximately a ten-fold increase in dosage to produce NAD depression comparable with that observation with those compounds containing a methyl end group.

3. Clinical antitumor activity and pharmacology of streptozotocin.

Streptozotocin has been administered to 106 patients with advanced malignancies. Therapeutic responses were observed in Hodgkin's disease, 7/16, lymphocytic lymphoma, 3/11, Burkitt's lymphoma, 1/12. and acute lymphocytic leukemia, 1/5. Four of nine patients with islet cell carcinoma, one insulin-secreting and one serotonin-secreting, responded, but eight patients with malignant carcinoid originating from the ileum failed to demonstrate a reduction in either urinary 5-hydroxyindole acetic acid or tumor mass. Additional evidence of drug activity was observed in two patients with melanoma and one of eight cases with sarcoma.

Two patients with "pancreatic cholera" and histologically proven hepatic metastases were treated with intra-arterial streptozotocin and both obtained a complete remission. When arterial infusions were compared to peripheral venous infusions in these two patients peripheral venous levels of streptozotocin were reduced by 50% and urinary excretion decreased by 33%. These pharmacologic studies indicate that the arterial route of administration increased tumor and/or hepatic extraction of streptozotocin and increased renal exposure to this nephrotoxic drug.

4. Prediction of clinical drug toxicity from animal data.

The section has been concerned with the ability of animal toxicologic studies to predict qualitative and quantitative toxicities encountered during clinical study of anticancer agents. Correlations between animal and human data have been made to allow physicians to better interpret toxicologic data and have been continually updated.

5. Procarbazine

A study has been completed to determine the extent the host drug metabolism can modify procarbazine such that its potent _in vitro_ oxidation hemolytic potential is ameliorated. A series of _in vitro_ experiments have clearly demonstrated that procarbazine is capable of oxidizing reduced glutathione. This in turn contributes to rapid disease in red cell sulfhydryl (-SH) groups. There is a secondary oxidation of oxyhemoglobin to form methemoglobin with red cell membrane damage and increased osmotic fragility. We demonstrated that this is effected by the ability of procarbazine to produce H_2O_2 in situ. Glucose-6-phosphate dehydrogenase (G-6-PD) deficient red cells are more sensitive than normal. Larger doses of parenteral procarbazine were administered to patients who received a cohart of chromium labelled G6PD deficient red cells (ABO Rh chomatible). No alteration of survival was noted. Furthermore, a series of procarbazine metabolites were examined for their _in vitro_ oxidative potential. The hydrazone which represents a minor metabolite was active, however, the major metabolites were totally inactive suggesting that rapid _in vitro_ conversion of the drug occurs to metabolites which lack erythrotoxic activity.

Honors and Awards

Dr. Schein

1. "Malignant Insulinoma: Mechanism of Action of Streptozotocin". Presented at Stanford University Medical Center, July 11, 1973.
2. "Structure Activity Relationships of Nitrosourea Antitumor Agents". Presented at Roswell Park Memorial Institute, Dec. 3, 1973.
3. "Biochemical and Kinetic Approach to Cancer Chemotherapy". Presented at Correlations between Internal Medicine and Basic Sciences. NIH, Med. 501, Dec. 5, 1973.
4. "Ara-Cytocine". Presented at George Washington University Medical Center, Pharmacology Course. Feb. 19, 1974.
5. "Prediction of Clinical Toxicities of Anticancer Agents". Presented at Symposium on Fundamental Cancer Research, Houston, Texas. Feb. 28, 1974.
6. "Structure-Activity Relationships for Diabetogenic Activity of Nitrosourea Compounds in Mice". Presented at Society of Toxicology. March, 1974.

7. "2-[3(2-Chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose, DCNU, (NSC 178248); A Water Soluble Nitrosourea with Reduced Bone Marrow Toxicity". Presented at Proceedings Am. Assoc. Cancer Res., March, 1974.

8. "Correlation with Extent of Depression of Pancreatic Islet Nicotinamide Adenine Dinucleotide (NAD". Presented at Proceedings of the Am. Fed. for Clin. Res., 1974.

9. "Burkitt's lymphoma Prediction of Early Relapse and Metabolic Complications of Therapy". Presented at, Proceedings Am. Society Clin. Oncology, #741, 1974.

Publications:

Dr. Schein

1. Schein, P.S., O'Connell, M.J., Blom, J., Hubbard, S., Magrath, I.T., Bergevin, P., Wiernik, P.H., Ziegler, J.L., and DeVita, V.T.: Clinical Antitumor Activity and Toxicity of Streptozotocin (NSC-85998). Cancer (in press).

2. Anderson, T., Schein, P.S., Jencks, J.A., and Binder, R.A.: The Nitroblue Tetrazolium (NBT) Dye Test in Determining Fever Source in Lymphoma. Cancer (in press).

3. Edelson, R.L., Kirkpatrick, C.H., Shevack, E.M., Schein, P.S., Smith, R.W., Green, I., and Lutzner, M.: Preferential Cutaneous Infiltration by Neoplastic Thymus-derived Lymphocytes: Morphologic and functional studies. Ann. Int. Med. (in press)

4. Schein, P.S.: The Prediction of Clinical Toxicities of Antitumor Drugs. In, Pharmacological Basis of Cancer Chemotherapy (in press).

5. Edelson, R.L., Schein, P.S., Lutzner, M.: Sezary Syndrome In, Current Dermatologic Management (in press).

6. Sponzo, R. W., Arseneau, J. C., And Canellos, G.P.: Procarbaxine Induced Oxidative Hemolysis: Relationship to in vivo erythrocyte survival. Brit. J. Haemat. (in press)

Serial No. NCI-6700
1. Medical Breast Cancer Service
2. Medical Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Clinical Program in Breast Carcinoma

Previous Serial Number: Same

Principle Investigators: Douglass C. Tormey, M.D., Ph.D., Joan M. Bull, M.D. and Paul P. Carbone, M.D.

Other Investigators: A.S. Ketcham, M.D., E.V. de Moss, M.D., J. Blom, M.D., M. Sears, M.D., E. Perlin, M.D., G. Falkson, M.D., P. Band, M.D., T. Pomeroy, M.D., D. Henson, M.D., J. Doppman, M.D., E. Jones, M.D., G. Johnston, M.D., M. Cohen, M.D., Ph.D., P. Chretien, M.D., R. Herberman, M.D., R. Oldham, M.D., first year clinical associates, second year clinical associates, P. Schein, M.D., E. Chu, M.D., J. Whang-Peng, M.D., R. Knazek, M.D., R. Everson, M.D., B. Thompson, M.D., P. Waalkes, M.D., Ph.D., R. Simon, Ph.D., S. Carter, M.D., and contractors

Cooperating Units: Surgery Branch, NCI, Radiation Branch, NCI, Pathology Branch, NCI, Office of the Associate Director for MO, NCI, Cancer Therapy Evaluation Branch, NCI, contractor hospitals, Medicine Branch, NCI, Diagnostic Radiology, NCI, Nuclear Medicine, NCI, Laboratory of Pathology, NCI, Laboratory of Chemical Pharmacology, NCI, Laboratory of Biochemistry, NCI, Experimental Biology Projects Section, NCI, Tumor Cell Biology Laboratory, NCI, Cellular and Tumor Immunology Section, NCI, Therapeutic Projects Section, NCI, and the Epidemiology Branch, NCI

Man Years:

Total: 10.3
Professional: 6.80
Other: 3.5

Project Description:

The clinical program in breast carcinoma by the Medical Breast Cancer Service involves three major areas of investigation:

Serial No. NCI-6700

I. Clinical Trials in Breast Carcinoma Patients
II. Ancillary Studies in Breast Carcinoma Patients
III. Extramural Activities in Breast Carcinoma Patients.

The Medical Breast Cancer Service was established in July 1972 and the clinical program was initiated in January 1973. It is responsible to the Office of the Associate Director, MO, Paul P. Carbone. The program is supported by one secretary, two technicians, two second year clinical associates, two first year clinical associates, and additional direct personnel support from the OAD, Experimental Biology Projects Section, and Epidemiology Branch, NCI in order to support the clinical program. The program has maintained an interdisciplinary approach to treatment with the Surgery Branch, Radiation Branch, and Pathology Branch at NCI. It operates from 4 beds on 13-East. Four one-half day clinics are maintained, including a joint Breast Problem Clinic with the Surgery Branch. Bed utilization has been 103%. Approximately 2500 clinic visits are projected for this fiscal year. The activities in each of the three major areas are outlined below.

I. Clinical Trials

A. Approach to the primarily diagnosed patient. 7XX

1. The Breast Problem Clinic is geared at evaluating tumor markers and other diagnostic techniques prior to biopsy.

2. Following radical mastectomy, patients with histopathologically positive axillary lymph nodes are entered into an NCI protocol comparing observation to cyclophosphamide, methotrexate, and 5-fluorouracil.

3. A protocol for the treatment of inoperable localized disease is under discussion between the Surgery Branch, Radiation Branch, and the Medical Breast Cancer Service.

B. Hormone Plus Chemotherapy Trials in Patients With a First Recurrence of Disease. These studies are designed to evaluate the role of chemotherapy as an adjunct to standard hormone therapy in patients presenting with their first recurrence of disease since mastectomy. These programs were designed with the cooperation of the Surgery Branch. 7XX

1. Between 5 and 15% of Stage I and II patients present with their first recurrence at the mastectomy site. These patients undergo an excisional biopsy to confirm the recurrence, and have been evaluated clinically to ascertain if there are metastatic foci; if there are they become candidates for studies listed below (2 and 3). If there is no clinical evidence of disease the patients then enter a protocol which compares oophorectomy to

oophorectomy plus combination chemotherapy in premenopausal patients, or observations against combination chemotherapy in postmenopausal patients.

2. Premenopausal patients with a measurable first recurrence enter a protocol in which they undergo a bilateral oophorectomy followed by observation for up to 12 weeks. If progressive disease occurs in this interval they enter the second recurrence program (See C). If a response or no change status is present at 12 weeks, they are then randomized to receive either combination chemotherapy or no further therapy. The latter group will receive the chemotherapy regimen when progressive disease develops.

3. Postmenopausal patients with a measurable first recurrence enter a protocol in which they initially receive diethylstilbesterol for 12 weeks. If progressive disease occurs in this interval they enter the second recurrence program (See C). If a response or no change occurs at 12 weeks, they are then randomized to receive either diethylstilbesterol alone or in combination with chemotherapy. The patients relapsing on the hormone alone arm will subsequently receive combination chemotherapy after progressive disease occurs.

C. Second Recurrence Investigations. 6X1

1. A protocol comparing cyclophosphamide, methotrexate, and 5-fluorouracil to cyclophosphamide, adriamycin, and 5-fluorouracil has had 56 entries as of March 15, 1974. Among the 32 evaluable patients there is evidence that the adriamycin containing arm is at least as active as the methotrexate containing arm. This study is expected to terminate in May or June 1974 and will be replaced by another protocol.

2. A Phase II protocol evaluating the combination of dibromodulcitol and adriamycin currently has 29 entries and is expected to close in May or June 1974. It will be replaced by another study.

3. A study evaluating dibromodulcitol alone as intermittent therapy has accrued 7 patients. Upon closure of the adriamycin-dibromodulcitol protocol patients will be entered primarily into the dibromodulcitol protocol at which time accrual will sharply increase. It is anticipated that this study will be replaced by another during the Summer of 1974.

4. Protocols evaluating medical adrenalectomy, oophorectomy, and hypophysectomy are due to be activated during the Summer of 1974. A protocol comparing the anti-estrogen Nafoxidine against Nafoxidine plus Fluoxymesterone has been activated and will be revised to substitute Tamoxifen, a less toxic anti-estrogen, for

the Nafoxidine in May or June 1974.

II. Ancillary Studies

Each of these programs is designed to provide either a better understanding of the disease process or to evaluate procedures which can be utilized early in the course of the disease. Since the clinical program was formally activated in January 1973 there is very little analyzed data from these programs at the present time; however, most of them will be ready for intensive analysis during the next 6 to 8 months.

A. Randomization Techniques. Breast carcinoma is a disease which is associated with a large number of prognostic factors. These necessitate a large stratification matrix in order to provide balance in each cell when randomization is performed. The usual techniques of randomization only assure balance within each stratification cell. In a two arm study it becomes possible to have 2 or 3 times as many patients on one arm as on the other, thus unduly prolonging the study. In cooperation with Dr. Richard Simon of the Biostatistical Information Systems Unit of the OAD, MO, we have designed a system which attempts to avoid this problem while still providing balance of the regimens within each cell. This system, which we have termed an "up-dated balance cell procedure," has been used successfully in several protocols and has now been designed so that it can be handled by a computer. As our primary protocols come to completion we will review this system for its validity.

B. Data Collection, Storage, Retrieval and Analysis

At present the usual method for performing clinically related studies involves hand collection of data from a patient's flow sheets and medical records with reference to each individual study. It would be considerably simpler if both the basic patient data and and individual study could be placed on a computer file. Appropriate cross indexing of this data would then allow numerous investigators to correlate their individual data with the patient's basic medical record. This would save innumerable man hours of searching patients' records. In cooperation with Dr. Richard Simon's group we have completed the basic forms which are necessary for this undertaking. It is anticipated that the computer programming will be completed during this calendar year. At that point sub-programs will be written to cross-index those pieces of information which are felt to be of interest. This is an on-going project which is being followed by other investigators for its usefulness in clinical research.

C. Chromosome Analysis

In cooperation with Dr. Jacqueline Whang-Peng and the Surgery Branch we are attempting to provide material serially on patients for chromosome analyses. This project is designed to further our understanding of the disease process and to evaluate the role of chemotherapy in the induction of chromosomal abnormalities.

D. Tumor Marker Analyses

In cooperation with Dr. Phillip Waalkes' laboratory we have supplied blood and urine samples from patients for analysis of tumor markers. The results of this program were reported to the American Society of Clinical Oncology, Inc. in March 1974 and they indicate that 65% to 70% of patients pre- and postoperatively, and 97% of patients with metastatic disease, will have elevations of one or more of the following markers: human chorionic gonadotrophin, carcinoembryonic antigen, or N^2,N^2-dimethylguanosine. We are continuing to evaluate new markers and will now look at the relationship of these markers to tumor burden, site of lesions, pathology, and response to therapy.

E. Pathologic Evaluation

Using a jointly arrived at pathology format all lesions are being routinely evaluated for a variety of histologic patterns. This information will be correlated with other clinical variables.

F. Radionuclide Scanning Procedures

The diagnostic importance of brain, liver-spleen, bone, and gallium scans in breast carcinoma has not been evaluated systematically. Evaluation of the breast proper by scanning techniques has not been performed. We have established with the Radionuclide Branch a standard series of scans to be performed on a routine sequential basis on our patients. The results of these procedures are being correlated with the clinical state to ascertain their capabilities relative to other clinical modalities. At the present time the data suggests that liver-spleen scanning may not be a very useful parameter, gallium scanning appears to be a good screening modality but unsuitable for follow up purposes. Bone scanning appears to be very valuable. The data in the other areas is too early at this time.

G. Hormone Analyses

We are measuring a variety of steroid hormone and hormone metabolites before and after chemotherapy in our patients. The results of these analyses are only now beginning to come back from the contracting laboratory and these will be analyzed over the next year.

H. Evaluation of Steroid Binding Proteins

Dr. Brad Thompson has been receiving tissue from the Surgery Branch and the Medical Breast Cancer Service for the analysis of steroid binding receptors. This data will be correlated with the response to hormone manipulations. It will also be looked at from the standpoint of changes in the receptor levels during the history of the disease.

III. Extramural Activities

The activities listed below have a direct bearing on the program of the Medical Breast Cancer Service in terms of either providing patient material or in integrating the NCI programs across varying branches.

A. Contract Programs 6X1, 7XX, 5XX

The National Naval Medical Center, Bethesda, and Walter Reed Army Hospital, Washington, are under contract to NCI. Douglass C. Tormey is project officer for these contracts. Both institutions take part in our protocol designs and utilization, thus shortening the duration of time required to complete our studies. To a lesser extent they participate in some of our ancillary study programs. In addition Walter Reed Army Hospital runs Phase II and III protocols in other disease categories.

Douglass C. Tormey has been working with Richard Simon to increase the efficiency of data collection for improved data analysis in the Phase II contracts of Stephen Carter in CTEB, and the Mayo Clinic Phase II contract under Douglass C. Tormey. The system which has been put into effect appears to work well for data collection and will shortly be programmed for computer analysis.

Dr. Geoffrey Falkson of Pretoria, South Africa and Dr. Pierre Band of Edmonton, Alberta both cooperate with the Medical Breast Cancer Service on a non-contractoral basis to participate in several of the chemotherapy studies.

The MBCS's affiliation with these cooperative groups and the Breast Cancer Task Force affords a unique opportunity to coordinate studies between various investigators.

B. Breast Cancer Task Force 1XX, 7XX

The Medical Breast Cancer Service participates in the Treatment Subcommittee of the Breast Cancer Task Force. Douglass C. Tormey and Paul P. Carbone, chairman of the subcommittee, have been involved in writing requests for proposals, site visits, and evaluations. Through this mechanism both Dr. Tormey and Dr. Carbone are project officers for several contracts.

C. Cooperative Group Affiliations 5XX, 5X1, 7XX

The Medical Breast Cancer Service is affiliated with Acute Leukemia Group B, in which Douglass C. Tormey is study chairman of two breast cancer studies. Douglass C. Tormey is co-chairman of the Breast Tumor Subcommittee of the Eastern Cooperative Oncology Group, and is co-author of the Metastatic Disease Protocol in that group.

Publications:

1. Bergevin, P.R., Tormey, D.C., and Blom, J.: Clinical evaluation of hexamethylmelamine (NSC 13875). Cancer Chemother. Rep. 57: 51-58, 1973.

2. Stutz, F.H., Tormey, D.C., and Blom, J.: Non-bacterial pneumonitis with multidrug antineoplastic therapy in breast carcinoma. CMA Journal 108: 710-714, 1973.

3. Stutz, F.H., Tormey, D.C., and Blom, J.: Hemangiosarcoma and pathologic rupture of the spleen. Cancer 31: 1213-1215, 1973.

4. Tormey, D.C., Bergevin, P., Blom, J., and Petty, W.: Preliminary trials with a combination of adriamycin (NSC 123127) and bleomycin (NSC 125066) in adult malignancies. Cancer Chemother. Rep. 57: 413-418, 1973.

Scientific Presentations

1. Bull, J.M., Tormey, D.C., Falkson, G., Perlin, E., Blom, J., and Carbone, P.P.: A comparison of adriamycin and methotrexate in combination chemotherapy regimens for metastatic breast cancer. Presented at the American Association for Cancer Research Meeting, March 1973.

2. Tormey, D.C., Waalkes, T.P., and Ahmann, D.L.: Multiple biologic markers as an aid to the evaluation of the status of patients with breast carcinoma. Presented at the American Society of Clinical Oncologists Meeting, March, 1973.

Serial No. NCI-6701
1. Medical Breast
   Service
2. Medical Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 to June 30, 1974

Project Title: Factors Controlling Lymphocyte Reactivity *in Vitro*

Previous Serial Number: Same

Principle Investigator: Douglass C. Tormey, M.D., Ph.D.

Other Investigators: Lynda Chiodetti, Helen Eliot, Robert Margolis, Robert Cates

Cooperating Units: None

Man Years:

Total: 3.8
Professional: 2.0
Other: 1.8

Project Description: 1XX

Objectives:

The normal physiologic controls of organized cell replication appear to be deranged in tumor growth. In tumor tissue there appears to be a greater probability of cellular replication as expressed in an abnormally high growth function compared to the normal tissue. This increased replication probability can also be expressed as a tendency for cells to leave the non-replicating G1 and G0 compartments to take part in one or more replication sequences. Physiologic "hormones" have been shown to inhibit or stimulate this process. The goal of this project is to attempt to isolate and identify similar entities for lymphocyte replication. The lymphocyte was chosen as a model for these interactions because human cells can be used, they can be purified, subpopulations can be identified, they exist *in vivo* in a prolonged G1 (?G0) state, can be selectively stimulated into parasynchronous growth moving sequentially through the cell cycle, the cell cycle transit times are known, and there is considerable background on the biochemistry of these cells following stimulation. The roles of lymphocytes in the immune response was an added attraction. In previous investigations my associates and I have identified a specific G1 requirement for transferrin by at least a subpopulation of these cells, confirmed the existence of an alpha-2 globulin containing fraction of human serum which is inhibitory for these cells, shown that two pig spleen extracts are inhibitory, and demonstrated the existence of a growth factor which is released by lymphocytes within 3 hours of culture.

Methods Employed:

A variety of chromatographic, precipitation, centrifugation, immunologic, and other techniques are utilized in the fractionation of the growth altering principles. Various tissue culture assays are used to assess the effects of these fractions.

Major Findings:

A factor released into the medium during the first few hours of lymphocyte culture in the absence of a mitogen has been shown to be subsequently required for the full expression of the action of a mitogen upon lymphocytes. This fraction has been found to be different from transferrin. It appears to be separable into 2 or 3 entities comprising at least one inhibitor and one or two growth promoting factors. Fractionation of this material is underway. Physiologic studies of its release and the cell requirements for this material is also underway. Progress has been made in attempts to purify lymphocytes into T and B subpopulations. A major problem in this area has been to effect the separation such that the purity is of very high grade and the cells are still viable for in vitro use. Some of these problems have been overcome and work continues in this area. The effect of various major steroids on lymphocyte growth are in the process of being defined. We are awaiting the renovation of our laboratory to complete these latter studies.

Significance to Biomedical Research and Program of the Institute:

This project has broad applicability to problems of altered cell growth, especially cancer and the immunologic system. The elucidation of molecular factors which physiologically control the differentiation of cells has direct potential application to therapy.

Proposed Course

Continued work on the fractionation of these factors is in progress, as well as physiologic studies concerning their action on the cell. We are continuing to attempt purification of T and B lymphocyte populations so that this can be scaled up for use in large in vitro studies. The future course of the work with steroids will be dependent upon the results obtained after the new laboratory is completed.

Scientific Presentations

1. Chiodetti, L.M., and Tormey, D.C.: Distinction between two lymphocyte growth promoting fractions. Presented at the American Association for Cancer Research Meeting, March 1973.

Serial No. NCI-3422
1. Pediatric Oncology Branch
2. Medical Oncology
3. Bethesda, Maryland, and
Kampala, Uganda

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Study and treatment of acute leukemia, solid tumors of childhood, hepatocellular carcinoma, and Kaposi's sarcoma

Previous Serial Number: Same

Principal Investigators: John L. Ziegler, M.D.

Other Investigators: Ian T. Magrath, Brigid G. Leventhal, Arthur S. Levine, Robert G. Graw, Geoffrey P. Herzig, Roger H. Halterman, Charles L. Vogel, Charles L. M. Olweny, and Sebastian K. Kyalwazi

Cooperating Units: Uganda Cancer Institute, Kampala, Uganda; Pathologic Anatomy Branch, NCI; Immunology Branch, NCI; Laboratory of Experimental Therapeutics, NCI; Surgery Branch, NCI; Radiation Branch, NCI; Departments of Surgery, Pathology and Medicine, Makerere University, Kampala, Uganda

Man Years:

Total:	6
Professional:	3
Other	3

Project Description:

Objectives:

The goals of the Pediatric Oncology Branch are to: (1) develop a diversified clinical service to facilitate the clinical and laboratory investigation of common childhood neoplasms; (2) explore the use of intensive high dose chemotherapy utilizing optimal supportive care methodology; (3) study various aspects of tumor immunology and immunotherapy; (4) investigate the pattern of infectious complications in patients with neoplasia and develop optimal methods for diagnosis, treatment, and prevention; (5) study and improve the collection, preservation and delivery of blood products (platelets, granulocytes and bone marrow) for patients with pancytopenia; (6) investigate viral oncogenesis *in vitro* using the adenovirus-SV40 hybrid; and (7) develop a comprehensive educational program responsive to Board training requirements for oncology fellows.

To achieve these goals, certain intramural reorganizational measures have been taken to identify three major areas of investigation. The establishment of the Chemoimmunotherapy, Experimental Hematology, and Infectious Diseases Sections have been achieved, and the activities and accomplishments of each Section will be reported separately. The physical consolidation of the clinical activities to Ward 2 East has improved communication within the Branch and has allowed the development of a coordinated clinical pediatric program. The opening of six additional beds on Ward 2B is scheduled for late fall, 1974. This area will house six laminar flow modules and will be fully equipped for intensive pediatric care.

The educational program this year has been stimulating and well attended. Tuesday lectures in oncology and Thursday discussions on patient problems have covered a wide range of topics. The Oncology Fellows also organized a series of seminars on pediatric oncology, and weekly lectures for the nurses. Dr. S. Hersh (NIMH) has held a weekly meeting with the clinical support staff (social workers, nurses, play therapists) to discuss the psycho-social problems of patients with chronic illness. The seminar led by Dr. Ken Artiss which explores various aspects of the doctor-patient relationship has also had a successful year. The Branch has also been instrumental in setting up a NIH-wide Pediatric Grand Rounds, and a visiting professor program has been established. Finally, a six-month Pediatric Nurse-Oncology Training Program is planned for September, 1974, which will provide a course curriculum with acceptable University accreditation.

The clinical and laboratory studies of acute leukemia will be described in the appropriate Section reports. In diversifying the clinical service, protocols have been prepared during the past year for the study of childhood solid tumors. Six patients with Burkitt's lymphoma have been treated with cyclophosphamide, vincristine and methotrexate combination, and all achieved complete remissions. This protocol further studies the role of reductive radiotherapy and maintenance immunotherapy using BCG and Raji cells. This study is coordinated with extramural study of Burkitt's lymphoma in Kampala, Uganda (vide infra). Two children with rhabdomyosarcoma have been treated with an intergroup protocol, and a child with Hodgkin's disease (stage IV) is under chemotherapy with MOPP. A protocol for metastatic neuroblastoma is now prepared which utilizes high-dose cytoxan, vincristine and DTIC in a dose-escalation study. All patients with solid tumors are being studied immunologically to determine (1) immunocompetence and (2) tumor-directed immunity. Future solid tumor studies will explore high-dose induction chemotherapy in patients given optimal hematologic supportive care and isolated in the laminar flow protected environment.

This office is also responsible for the extramural activities of the Uganda Cancer Institute, Contract No. PH43-67-1343, directed by Professor S. Kyalwazi and C. Olweny at Makerere University, Kampala. Transitional adjustments during the past year (departure of Drs. Magrath and Vogel, arrival of Dr. Olweny) were smoothly accomplished, and a recent progress report from the Institute indicates that patient accrual and followup are proceeding normally.

The Lymphoma Treatment Center, devoted to the treatment of Burkitt's tumor, Hodgkin's disease, and other childhood tumors has been fortunate in obtaining the services of Dr. E. K. Mbidde, a Ugandan pediatrician who was trained at the Institute as a student. The protocol studying BCG immunotherapy in Burkitt's tumor was completed in August, 1974, and showed that while BCG potentiated delayed hypersensitivity reactions during remission, relapse occurred with the same frequency (11/22) as in control patients not receiving BCG (10/20). Immunological studies showed that skin test reactivity reflected disease activity, and cutaneous anergy appeared a result, rather than a cause of tumor presence. Other important observations included: (1) the beneficial effect of extensive surgical removal of abdominal tumor; (2) the T and B nature of Burkitt lymphoma cells studied in vitro; (3) the observation of the development of Burkitt's tumor in a child with prior Hodgkin's disease in remission and the concomitant rise of antibody to EBV early antigen; (4) the utility of fine needle aspiration in the rapid diagnosis of malignant lymphoma; (5) further confirmation and extension of cell kinetic data in Burkitt's lymphoma. These and other studies are now either published or in preparation, and are listed in the bibliography.

Since Dr. Magrath's departure, Dr. Olweny has undertaken the followup of Burkitt patients treated under previous protocols, and has designed a new randomized study comparing multiple-dose cyclophosphamide with combination chemotherapy (cyclophosphamide, vincristine, methotrexate); prophylactic cranial-spinal irradiation of patients presenting without malignant pleocytosis is administered in a further randomized trial. Thus far 14 patients have entered the protocol, but results are too preliminary to report.

The Solid Tumor Center has reactivated studies in hepatocellular carcinoma, concentrating on (1) the utility of hepatic artery ligation in stage 1 disease; and (2) the use of adriamycin 75 mg/m^2 in patients with inoperable tumor. In the first study, 3/6 patients died postoperatively and the remainder are alive 12, 16, and 18 following surgery, and are clinically well. The adriamycin study has revealed 10/10 responses (2 complete and 8 partial), with complete remissions lasting 7 and 8 months respectively. This is the first drug demonstrating beneficial activity in this disease, and confirmatory data are awaited from other trials now in progress. Collaborative studies of alpha-feto protein are still conducted, and show decreases in concentration which parallel the clinical response.

Studies in Kaposi's sarcoma constitute the remainder of the Solid Tumor Center activities. A trial begun in 1972 which examined the additive effect of DTIC to the vincristine/actinomycin D combination is now completed and in preparation for publication. Of 42 patients randomized the complete response rates were (DTIC) 81% and (no DTIC) 57% respectively. All remaining patients exhibited partial responses, and the groups were comparable with respect to clinical type and histological classification. Data on remission durations are not yet available but the median will be in excess of 12 months. The DTIC/vincristine/actinomycin D combination was extremely well tolerated and now constitutes the treatment of choice for Kaposi's sarcoma. It is now planned to add Bleomycin to the combination, which is an active non-myelosuppressive agent in this disease.

The successful continuation of the clinical activities of the Uganda Cancer Institute augur well for its future as an integral part of the Makerere Medical School. The emphasis will remain on the careful conduct of randomized phase II and III clinical trials, patient surveillance, and collaborative studies with investigators abroad.

Of the variety of collaborative investigations underway, the work of Dr. Sol Spiegelman deserves mention. He and his colleagues found that 8% of Ugandan Burkitt's lymphomas contained reverse transcriptase and RNA which hybridizes with a DNA probe prepared from Rauscher leukemia virus. They also reported unique DNA sequences in the tumor tissue of patients with Hodgkin's disease and Burkitt's lymphoma.

Other collaborative studies in progress include: (1) HLA phenotypes in Burkitt's lymphoma (Bodmer, Oxford); (2) EBV antibody in Burkitt's lymphoma (Henle, Philadelphia); (3) Anti-Membrane antibodies in Burkitt's lymphoma (Klein, Stockholm); (4) Tumor markers (Waalkes, NIH); (5) G6PD tumor markers (Fialkow, Seattle); (6) EBV excretion in saliva (Niederman, New Haven); (7) Metabolic studies in Burkitt's lymphoma (Schein, NIH); and (8) West Nile EBV Seroepidemiology project (DeThe, Lyons).

In summary, the clinical and investigational activities of the Pediatric Oncology Branch are now changing in scope and content. The priority effort lies in the development of treatment programs for childhood solid tumors, utilizing the therapeutic advances achieved in acute leukemia, and emphasizing a combined modality approach. The clinical diversification will provide a broadened training program for Oncology Fellows, where the emphasis will be a high standard of medical care and on the application of biomedical research techniques to clinical problems. Studies in acute leukemia will continue where research will be directed primarily to immunotherapy and supportive care.

Honors and Awards:

Invited Lectures:

John L. Ziegler, M.D.

1. Tropical Splenomegaly Syndrome. Georgetown University. July, 1973.
2. Burkitt's Lymphoma. Johns Hopkins University. December, 1973.
3. Burkitt's Lymphoma. University of South Carolina. April, 1974.
4. Tumor Surveillance. Tennessee Medical Association. April, 1974.
5. Burkitt's Lymphoma. New England Deaconess Hospital. May, 1974.
6. Experimental Therapy in Childhood Cancer. American Cancer Society Conference on Childhood Cancer. Dallas, Texas. May, 1974.
7. Burkitt's Lymphoma. Conference on Spontaneous Regression in Cancer. Johns Hopkins University. May, 1974.

Appointments:

1. NCI Immunotherapy Coordinating Committee. November, 1973.
2. National Cancer Plan Participant. February, 1974.
3. Medical Board, NIH Clinical Center. March, 1974.

Certification:

1. Diplomate. American Board of Internal Medicine. October, 1973.
2. Diplomate. Subspecialty of Medical Oncology. American Board of Internal Medicine. October, 1973.

Publications:

1. Ziegler, J.L. and Burchenal, J.H.: Treatment of Burkitt's tumor. In Lymphosarcoma and Related Diseases, Molander and Pack (eds.), Charles C. Thomas, New York, 1973.
2. Ziegler, J.L.: Burkitt's lymphoma. In Cancer Medicine, Holland, J.M. and Frei, E.T. (eds.), Lea and Febiger, 1973.
3. Ziegler, J.L., Voller, A., and Ponnudurai, T.: Malarial antibodies in tropical splenomegaly syndrome in East Africa. Trop. Geogr. Med. 25: 282, 1973.

4. Kufe, D., Magrath, I.T., Ziegler, J.L., and Spiegelman, S.: Burkitt's tumors contain particles encapsulating RNA-instructed DNA polymerase and high molecular weight virus-related RNA. Proc. Nat. Acad. Sci. 70: 737, 1973.

5. Ziegler, J.L.: Cryoglobulinemia in tropical splenomegaly syndrome. Clin. Exp. Immunol. 15: 65, 1973.

6. Woodruff, A. W., Ziegler, J.L., Hathaway, A., and Gwata, T.: Anemia in African trypanosomiasis and tropical splenomegaly syndrome in Uganda. Trans. Roy. Soc. Trop. Med. Hyg. 67: 329, 1973.

7. Magrath, I.T.: Fine-needle aspiration - the diagnosis of childhood malignant tumors in Uganda. Brit. J. Cancer. 28: 477-487, 1973.

8. Magrath, I.T., Ziegler, J.L., and Templeton, A.S.: A comparison of clinical and histological features of childhood malignant lymphoma in Uganda. Cancer. 33: 285, 1974.

9. Ziegler, J.L.: Burkitt's lymphoma. In Pathobiology Annual, Ioachim, H.L. (ed.), Appleton Century Crofts, New York, 1974.

10. Ziegler, J.L.: Treatment of Burkitt's lymphoma. In Modern Radiotherapy, Buttersworth, London, 1974.

11. Magrath, I.T., Ziegler, J.L., and Templeton, A.C.: A comparison of clinical and histopathological features of childhood malignant lymphoma. Cancer. 33: 285-294, 1974.

12. Magrath, I.T. and Ziegler, J.L.: BCG immunotherapy in Burkitt's lymphoma. In Semaine de Cancerologie, Springer-Verlag, 1973 (in press).

13. Bluming, A.Z., Vogel, C.L., and Ziegler, J.L.: Screening of systemic adjuvants of immunity. In Semaine de Cancerologie, Springer-Verlag, 1973 (in press).

14. Ziegler, J.L. and Magrath, I.T.: BCG immunotherapy in Burkitt's lymphoma. J. Nat. Cancer Inst. (in press).

15. Iversen, O., Iversen, U., Ziegler, J.L., and Bluming, A.Z.: Cell kinetics in Burkitt's lymphoma. Europ. J. Cancer (in press).

16. Ziegler, J.L.: Chemotherapy and survival of Burkitt's lymphoma. Oncology (in press).

17. Ziegler, J.L.: The usefullness of controlled clinical trials in cancer treatment. Oncology (in press).

18. Magrath, I.T.: Fine needle aspiration in the diagnosis of various childhood malignant tumours. Brit. J. Cancer (in press).

19. Magrath, I.T.: The Diagnosis and management of Burkitt's lymphoma in Uganda. I. Diagnosis. Uganda Med. J. (in press).

20. Magrath, I.T., Lwanga, S., Carswell, W., Harrison, N.: Surgical reduction of tumour bulk in the management of abdominal Burkitt's lymphoma, Brit. Med. J. (in press).

21. Magrath, I.T.: The diagnosis and management of Burkitt's lymphoma in Uganda. I. Diagnosis. Uganda Med. J. (in press).

22. Magrath, I.T.: Viruses and Human Neoplasia. Makerere Med. J. (in press).

23. Magrath, I.T.: Burkitt's lymphoma: A, B or T cell tumour? Europ. J. Cancer (in press).

24. Magrath, I.T., Mugerwa, J., Bailey, I., Olweny, C., and Kiryabwire, Y.: Intracerebral Burkitt's lymphoma - Pathology, clinical features and treatment. Quart. J. Med. (in press).

25. Magrath, I.T., Immunosuppression in Burkitt's lymphoma. I. Cutaneous reactivity to recall antigens, alleviation induced by a tumour burden and by BCG administration. Int. J. Cancer (in press).

Serial No. NCI-6308
1. Biostatistical Information
   Systems Unit
2. Medical Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biostatistical Information Systems Unit (BISU)

Previous Serial Number: Same

Principal Investigator: Richard Simon, Ph.D.

Other Investigator: None

Cooperating Units: Medicine Branch, MO, DCT, NCI
Medical Breast Cancer Service, MO, DCT, NCI
NCI-VA Medical Oncology Branch, MO, DCT, NCI
Pediatric Oncology Branch, MO, DCT, NCI
Immunology Branch, DCBD, NCI
Surgery Branch, DCBD, NCI

Man Years:

Total: 1
Professional: 1
Other: 0

Project Description:

The Biostatistical Information Systems Unit was established during FY 1972 in order to utilize statistical methodology and computer technology to maximize knowledge obtained from clinical trials and other research carried out within the Medical Oncology Area of the Division of Cancer Treatment. In addition to direct participation in the design and analysis of clinical trials and other experiments, the Unit conducts research in statistical methodology in support of the above goals. A list of projects in which the Unit is actively involved follows.

1. Participation in the design, monitoring, and analysis of clinical trials. Development of disease and protocol specific evaluation forms for the analysis of clinical trials. Monitor contract for the abstraction and computerization of data concerning patient pretreatment characteristics, treatment administered, response, complications, and follow-up information. Development of computer systems and production of reports to assist in the monitoring of clinical trials. Thorough analysis of trial results in disseminated bronchogenic carcinoma, non-Hodgkin's lymphoma, ovarian carcinoma, acute leukemia, and carcinoma of the breast.

Effective protocol monitoring and data handling is of great importance in multi-branch or multi-institution studies. In the past year we have developed an effective system of data handling and protocol monitoring for the cooperative melanoma study. Protocol specific forms have been developed and are in use. These forms include a staging form, a follow-up form for each clinic visit, an end-of-therapy form, and immunology laboratory forms. All data is computerized with useful reports generated. All patients are followed to death, and the BISU performs close protocol monitoring to ensure that the proper follow-up examinations are performed at the proper intervals. The system is fully operational. A similar, though even more comprehensive, data handling system has been developed for the studies of the Medical Breast Cancer Service.

2. Statistical collaboration in the design and analysis of retrospective clinical and laboratory studies. Retrieval of data from computer files and creation of computer files for these studies. Though there have been numerous statistical consultations, these have included: (a) Development of a new clinical staging system for Burkitt's lymphoma and evaluation of the prognostic importance of clinical and immunological parameters; (b) Development of a new clinical staging system for Hepatocellular Carcinoma; (c) Evaluation of the relationship of hemoglobin type on immunoglobulin levels in Ugandan children; (d) Evaluation of the effectiveness of methotrexate as a surgical adjuvant for head and neck cancers; (e) Evaluation of the duration of initial complete remission as a predictor of subsequent survival for children with ALL; (f) Evaluation of the importance of duration of maintenance therapy for children with ALL; (g) Analysis of long second complete remissions in children with ALL; (h) Analysis of serum factors and blastogenic response to leukemia cells in patients with ALL; (i) Evaluation of the relationships between cytogenetics and survival in patients with acute leukemia; (j) Analysis of the effects of chemotherapeutic drugs on in-vitro growth of bronchogenic carcinoma cells; (k) Development of a predictive model for relating blood sugar and protein levels to tumor burden in carcinoma of the breast; (l) Evaluation of the relationship between HLA type and carcinoma of the breast.

3. Abstraction, computerization, and analysis of data concerning the efficiency of procurement and effectiveness of infusion of granulocytes and platelets. Maintenance and report generation for computerized serum bank file. Development of a system for generating computerized mailings to all donors.

4. Participation in the design and analysis of Phase II clinical trials performed on contract. Development of a uniform computerized data reporting and analysis system in use for Phase II clinical trials of carcinoma of the breast performed on contract to the Division of Cancer Treatment. Data is updated bimonthly and analyzed centrally. Summary of analyses are distributed bimonthly to individuals involved in monitoring these contracts. Analysis consists of evaluation of response and toxicity to chemotherapy, prognostic value of proposed discriminants,

impact of study design upon survival, cross-resistance evaluations, etc. This system is fully operational with data received from 4 institutions.

5. Development and maintenance of a computerized file of all world-wide clinical trials in carcinoma of the breast supported by the National Cancer Institute. The file includes a description of the patient selection, treatment programs, stratifications used, date the trial began, ended, last reported, and a summary of the results. This file is maintained in collaboration with the Medical Breast Cancer Service. A similar file of studies in bronchogenic carcinoma is being developed in collaboration with the coordination office for the study of lung cancer.

6. Interfacing between the investigators of the Medical Oncology Area and the computer systems and personnel of the Clinical Center and Division of Computer Research and Technology in order to utilize computerized data available and to eliminate duplication of effort. Obtain retrievals from centralized computer files and develop computer programs to utilize the centralized files for our purposes. In the past year we have created retrievable subfiles of clinical laboratory data for all patients with melanoma or carcinoma of the breast. We have also tied into the computerized admission and discharge system of the Clinical Center which enables us to generate a report of all admissions and discharges by branch, date, sex, age, admissions diagnosis, etc. This report helps us to monitor protocols, and will be of value to the cooperating branches for administrative purposes.

7. Preparation of stratified randomization schemes and performance of randomizations for clinical trials. The new method of stratified randomization mentioned in reference (3) has been implemented here in our computer time sharing system. It is being used for all randomized studies of the Medical Breast Cancer Service. This new methodology permits us to ensure balance among many more stratification variables than do the older methods.

8. Development and application of methodology for the thorough analysis of cell kinetics experiments in human and animal systems. Computer programs developed are being utilized in research institutions throughout the world. We have been analyzing here the effect of chemotherapy on the growth kinetics of rat hepatoma 3924A.

9. Review of proposals for the procurement of computer equipment and related systems. Review of the impact on the Medical Oncology Area of the proposed Technion Hospital Information System for the Clinical Center. Review of proposed supplements to our contract with Dr. Terasaki for development of a comprehensive computerized system for the selection of platelet donors.

10. Project management of MO-DCT contract with Automation Industries for computer and data abstraction support. Project management of Breast Cancer Task Force (BCTF) contracts with NYU Medical Center and Rush-Presbyterian Hospital for cell kinetics studies of patients with Carcinoma of the Breast. Proposal review and site visit evaluations for proposed studies of the BCTF Treatment Committee, and for DCT contracts concerning protected environments and prophylactic antibiotics.

11. Statistical Research:

    a. Investigation of sequential termination and treatment assignment rules for the development of clinical trials which require fewer patients and allocate fewer patients to the inferior treatment than is accomplished by traditional formulations.

    b. Development and evaluation of new methods of allocating treatments to patients for clinical trials with few patients and many stratification variables. Traditional methods may result in considerable imbalance of stratification variables for such situations.

    c. Development of mathematical model for planning optimal schedules for combining modalities or drug combinations in the treatment of primary or metastatic carcinoma of the breast. The predictions of this model have serious implications upon schedule design and are being evaluated with past data, planned experiments in a rat mammary tumor, and planned clinical trials in human metastatic disease.

Publications

1. Leventhal, B. G., Simon, R., Henderson, E. S., and Ziegler, J. L.: The relation of duration of first remission to survival. In Fliedner, T. (ed.): Prognostic Factors in Human Acute Leukemia. Germany, Pergamon Press, 1973, (in press).

2. Simon, R., Weiss, G. H., and Hoel, D. G.: Sequential analysis of binomial clinical trials. Biometrika (in press).

3. Pocock, S. J. and Simon, R.: Sequential treatment assignment with palancing for prognostic factors in the controlled clinical trial. Biometrics (in press).

4. Graff, K. S., Simon, R., Yankee, R. A., DeVita, V. T., and Rogentine, G.M.: HL-A antigens in Hodgkin's disease: Histopathologic and clinical correlations. J. Nat. Cancer Inst. (in press).

Serial No. NCI-6810
1. Chemoimmunotherapy Section
2. Pediatric Oncology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Chemo-immunotherapy

Previous Serial Number: Same

Principal Investigator: Brigid G. Leventhal, M.D.

Other Investigators: Kenneth L. Sacks, M.D., Roger H. Halterman, M.D., Philip O. Cohen, M.D., John L. Ziegler, M.D., Werner A. Bleyer, M.D., Richard A. Bender, M.D., Dean L. Mann, M.D., Ronald B. Herberman, Richard M. Simon, M.D., G. Johnson, M. J. Sutherland, M. A. Rankin, S. Triem, N. Keyes, R.N.

Cooperating Units: Immunology Branch, NCI
Laboratory of Chemical Pharmacology, NCI
Cell and Tumor Immunology Section, LCB, NCI

Man Years:

Total: 10 1/2
Professional: 5 (excluding 1st year Clinical Associate)
Other: 5 1/2

Project Description:

Objectives:

1) To detect, characterize and purify specific tumor antigens in acute leukemia.

2) To assess the effect of chemotherapy and immunotherapy on the immune response in general.

3) To assess the effect of chemotherapy and immunotherapy on the specific response to tumor antigen.

4) To assess pharmacology of MTX in blood and CSF.

5) To devise therapeutic protocols which will allow maximal tumor cell kill with drugs while leaving intact effective elements of the anti-tumor immune response.

Progress:

A. Characterization of leukemia antigens and patient reactivity to these antigens during chemoimmunotherapy.

1. Humoral response. Patients with previously treated ALL in relapse were induced into remission with vincristine, prednisone, daunomycin and asparaginase and then randomized to maintenance with monthly COAP alone or COAP plus injections of RAJI cells, a Burkitt lymphoma derived tissue culture cell line which shares a leukemia-associated antigen with acute leukemia blast cells. Seven patients received COAP alone with a median remission duration of 150 days; 7 received COAP + RAJI with a median remission duration of 135 days. Four of 7 patients receiving RAJI injections developed antibody which was cytotoxic in a complement dependent system to acute leukemia blast cells. In a lymphocyte dependent system, no antibody was detected against acute leukemia cells although antibody against RAJI was seen. The patients' immune response seems to differ from the RAJI sensitized rabbits which developed both complement and lymphocyte dependent antibody. The mechanisms for these differences are being investigated.

Serologic detection of leukemia antigens is now being reported from many laboratories. Since our group was a pioneer in this field, we organized a workshop on this subject in collaboration with the Immunology Branch. Distinguished scientists from the U.S. and abroad attended and further workshops are planned.

2. Cellular response. Skin test response to autologous tumor appears to be associated with remission status and therefore good prognosis. Analysis of leukocyte culture responses, however, suggest that a positive response *in vitro* may reflect persistence of tumor. Blocking by relapse serum of the MLC response to autologous tumor was seen in only 1 of 18 patients studied and did not correlate with prognosis.

B. Clinical studies in leukemia.

1. Combined chemoimmunotherapy for ALL (NCI protocol 72-1). Sixty-two evaluable patients have been entered on this protocol of whom 57 have achieved remission. After consolidation all patients receive cyclic chemotherapy for 4 of 6 months for three years. In the other two months they receive either BCG and allogeneic leukemia cells or twice weekly methotrexate. A third arm, five-day courses of methotrexate, was dropped because of toxicity and variability in administration of drug from patient to patient. Thus far relapse has occurred in 4/18 patients on BCG; 4/17 on twice weekly methotrexate; and 4/12 on five-day methotrexate. There is slight evidence of improved cellular reactivity to antigens in the BCG group. Resistance to infection is being evaluated.

2. Prognostic factors. Analysis of survival as a function of duration of initial remission was performed. This allowed us to define a poor risk group of patients, with short initial remissions in whom experimental therapy would reasonable be performed early in the course of their disease. In addition, a group of five patients with second remissions lasting more than five years were described. All of these patients had completed scheduled therapy on earlier protocols and enjoyed at least six months of unmaintained remission prior to relapse.

C. Meningeal leukemia

1. Thirty-one patients with meningeal leukemia were randomized to chemotherapy with intrathecal methotrexate with and without 2400 r to the cranial vault. Remission duration was significantly longer (234+ days) in the group receiving MTX alone than in those receiving MTX + x-ray (125 days). A spectrum of methotrexate toxicity was seen after intrathecal administration ranging in severity from chemical arachnoiditis which was common (17 patients) through transient paresis (1 patient) to encephalopathy (2 patients). These toxicities were as common in patients receiving preservative-free MTX as in those receiving MTX with preservative and therefore should be ascribed to direct toxic effects of MTX itself or its breakdown products rather than to preservative.

2. After completion of the above studies a patient developed paraplegia following intrathecal methotrexate administration. It was possible to correlate this toxicity with abnormally high CSF MTX levels. The reason why this patient's levels were high is not clear, but it is proposed that intrathecal medication should not be administered on a per meter square basis since the volume of CSF is not proportional to the size of the rest of the body.

3. Currently patients with meningeal leukemia are having Ommaya reservoirs inserted and are being treated with MTX either in conventional pulsed doses or with frequent doses throughout a prolonged period to maintain a constant concentration over a standard time period. Seven patients have been entered on this protocol with a median remission duration in excess of 239+ days. Intensive systemic therapy is also given on this protocol in an attempt to totally eradicate established CNS disease.

Conclusions:

Tumor associated antigens in ALL can evoke a cytotoxic reaction in human patients after immunization with RAJI cells. To date, however, immunotherapeutic manipulations have not been effective in prolonging remission duration. A positive lymphocyte response to tumor *in vitro* may reflect a persistence of tumor *in vivo* rather than a destructive *in vivo* response. Prognosis for individual patients is best determined by analyzing the character of their initial remissions. In a small group of patients who relapsed after an unmaintained remission of at least six months, long (over five years) second remissions have been obtained. Analysis of drug levels in blood and spinal fluid and of drug sensitivity of blast cells may allow us to tailor our therapy more appropriately to the individual patients.

Proposed course of the project:

A major problem is the analysis of those elements in the immune response which are associated with and responsible for a good prognosis in malignant disease. To this end, extensive study of the immune response, both to common antigens and to tumor-associated antigens will be continued in all patients. The leukemia-associated antigen found on RAJI cells is of particular interest, since this might represent a good source of cells for immunotherapeutic vaccination programs. Monitoring of drug levels as well, both in leukemia cells and in body fluids will be studied to increase our knowledge in the rational use of these agents.

Honors and Awards:

Brigid G. Leventhal, M.D.

1. Federal Woman's Award, 1974.

2. Judge, Westinghourse Science Talent Search, 1974.

Invited Lectures and Chairmanships:

1. Immunotherapy. General Practice Section. D.C. Medical Society, June 1973.

2. Effect of Chemotherapy on the Immune Response. Children's Cancer Group A, June, 1973.

3. Co-chairman, Immunology Section, Conference on Prognosis in Acute Leukemia. Ulm, Germany, October, 1973.

4. Co-chairman, Leukemia Antigen Workshop, NCI. November, 1973.

5. Co-chairman, Leukemia Section, American Society Hematology Meeting. December, 1973.

6. Histochemistry in Clinical Hematology. Faculty of Histopathology, AFIP, January, 1974.

7. Seminar on Immunotherapy. Department of Pharmacology. George Washington School of Medicine. April, 1974.

8. Effect of Chemotherapy on the Immune Response in Acute Leukemia. Tumor Board. Medical College of Virginia, Richmond, Virginia, April, 1974.

9. Panelist, Nursing Section IV. Development of a Pediatric Nurse Oncology Training Program. COA meeting, April, 1974.

Publications:

1. Cullen, B.F., Chretien, P.B., Leventhal, B.G.: The effect of lignocaine on PHA stimulated human lymphocyte transformation. Brit. J. Anaesth. 44: 1247-1252, 1973.

2. Duttera, M.J., Bleyer, W.A., Pomeroy, T.C., Leventhal, C.M. and Leventhal, B.G.: Irradiation, methotrexate toxicity and the treatment of meningeal leukemia. Lancet ii: 703-706, 1973.

3. Halterman, R.H., Johnson, G.E., and Leventhal, B.G.: Storage method for leukemic blast cells. NCI Monograph 37: 149-152, 1973.

4. Lippman, M.E., Halterman, R.H., Leventhal, B.G., Perry, S., and Thompson, E.B.: Glucocorticoid-binding proteins in human acute lymphoblastic leukemic blast cells. J. Clin. Invest. 52: 1715-1725, 1973.

5. Falchuk, Z.M. and Leventhal, B.G.: Budd Chiari syndrome in a patient with paroxysmal nocturnal hemoglobinuria. Am. J. Dig. Dis. 18: 900-904, 1973.

6. Char, D.H., LePourhiet, A., Leventhal, B.G., and Herberman, R.B.: Cutaneous delayed hypersensitivity responses to tumor associated and other antigens in acute leukemia. Int. J. Cancer 12: 409, 1973.

7. Mann, D.L., Ulmer, C., LePourhiet, A., Leventhal, B.G., Halterman, R.H.: Studies on the immunologic reactivity of sera from acute leukemia patients. Oncology 17: 1-9, 1973.

8. Bleyer, W.A., Draje, J.C., and Chabner, B.A.: Neurotoxicity and elevated cerebrospinal fluid methotrexate concentration in meningeal leukemia. New Eng. J. Med. 289: 770-773, 1973.

9. Bryan, J.H., Henderson, E.S., and Leventhal, B.G.: Cytosine arabinoside and 6-thioguanine in refractory acute lymphocytic leukemia. Cancer 33: 539-544, 1974.

10. Leventhal, B.G., Simon, R.H., Henderson, E.S., and Ziegler, J.L.: The relation of duration of first remission to survival. In Fliedner, T. (ed.): Prognostic Factors in Human Acute Leukemia. Germany, Pergaman Press, 1973 (in press).

11. Herberman, R.B., Char, D.H., Oldham, R.K., and Leventhal, B.G.: The prognostic value of studies of specific cell-mediated immunity in acute leukemia. In Fliedner, T. (ed.): Prognostic Factors in Human Acute Leukemia. Germany, Pergaman Press, 1973 (in press).

12. Leventhal, B.G., Cohen, P., and Triem, S.C.: The effect of chemotherapy on the immune response in acute leukemia: A review. Israel J. Med. Sci. (in press).

13. Bryan, J.H., Johnson, G.E., and Leventhal, B.G.: The effect of autologous serum on lymphocyte response to human leukemia cells. Blood (in press).

14. Herberman, R.B., Char, D., Oldham, R., Levine, P., Leventhal, B.G., and McCoy, J.L.: Cell-mediated immunity in human acute leukemia. Proceedings of the VI International Symposium on Comparative Leukemia Research (in press).

15. Leventhal, B.G., Johnson, G.E., Bryan, H.B., and Halterman, R.H.: Speculation on the significance of the in vitro lymphocyte response to autologous tumor. In Fliedner, T. (ed.): Prognostic Factors in Human Acute Leukemia. Germany, Pergaman Press, 1973 (in press).

Serial No. NCI-6820 (c)
1. Experimental Hematology Section
2. Pediatric Oncology Branch, MO
3. Bethesda, Maryland

PHS-NIH
Individual Progress Report
July 1, 1973 through June 30, 1974

Project Title: Blood Component Transfusion and Bone Marrow Transplantation

Previous Serial Number: NCI-3415, NCI-6301, NCI-3426

Principal Investigators: Robert G. Graw, Jr., M.D., and Geoffrey P. Herzig, M.D.

Associate Investigators: Malcolm I. Bull, M.D., Julian Decter, M.D., Hans-Peter Lohrmann, M.D., Jean Charles Kauffmann, M.D, Ronald A. Yankee, M.D., Edward S. Henderson, M.D., Brigid G. Leventhal, M.D., G. Nicholas Rogentine, M.D., Gerhard R.F. Kreuger, M.D., John Neefe, M.D., Joan M. Bull, M.D., Costan Berard, M.D., Harvey Gralnick, M.D., Jacqueline Whang-Peng, M.D., Mary H. McGinniss, Thomas C. Pomeroy, M.D., David Johnson, D.V.M., Paul I. Terasaki, Ph.D, David Dale, M.D., Charles H. Kirkpatrick, M.D., Charles Bowles, Ph.D, Gary Peck, M.D., Michael Blase, M.D., Richard Hirshman, M.D., Richard Simon, Ph.D, Regina Dowling, R.N., Ruth Estabrook, R.N., Joanne Bemen, R.N., Louise Osborne, R.N., Carole Graw, Shirley House, Ligita Novikovs, Frank Stout, Daniel Dean, William Penland, Thomas Gray, Arthur Sanford.

Cooperating Units: Medicine Branch, NCI; Immunology Branch, NCI; Laboratory of Pathology, NCI; Hematology Service, Clinical Pathology, Clinical Center, NIH; Blood Bank, Clinical Center, NIH; Anesthesiology Department NIH; National Institute of Allergy and Infectious Disease, LCI; Department of Surgery, University of California at Los Angeles; Mary Imogene Bassett Hospital, Cooperstown, New York; Department of Medicine and Surgery, Division of Research Services, NIH; Plasmapheresis Unit, NCI; Dermatology Branch, NCI; Radiation Branch, NCI; Case Western Reserve, University School of Medicine, Cleveland, Ohio; Armed Forces Radio-Biology Institute, Bethesda, Maryland; Microbiological Associates, Bethesda, Maryland; Hazelton Laboratories, Falls Church, Virginia; ABCOR Corporation, Boston, Massachusetts; Oakridge National Laboratories, Oakridge, Tennessee; Community Blood Service, Baltimore, Maryland.

Man Years:

Total: 10
Professional: 6
Other: 4

Project Description:

Objectives:

1) The provision of effective blood component transfusion therapy for patients with depressed bone marrow function, with emphasis on the development of improved procurement and transfusion techniques for blood platelets and granulocytes.

2) Evaluation of histocompatibility antigen variables as they govern transfusion responsiveness in patients alloimmunized to transplantation antigens.

3) Assessment of the optimal methods for obtaining and maintaining allogeneic, autologous and isogeneic bone marrow grafts.

4) Examination of the effectiveness of combined modality treatment (chemotherapy, radiotherapy, immunotherapy and supportive care) in large animals with spontaneous neoplasms (canine lymphosarcoma).

5) Utilization of preclinical animal models (monkey and dog) to answer questions pertaining to clinical bone marrow transplantation.

Methods Employed:

1) Transfusions of platelets and leukocytes in the Clinical Center, NIH, have continued to be the major means of evaluation of new transfusion techniques. Seven thousand donors who have been HL-A typed form the available donor pool. A contract has been established for the plateletpheresis of selected donors thereby enlarging the capability for delivering selected (non-random) blood products for patients requiring platelet and granulocyte transfusions.

2) A limited effort in allogeneic bone marrow transplantation has been continued for terminal leukemic patients.

3) Pre-clinical animal model systems (primate and canine) have been utilized for testing transfusions, transplantation, and experimental techniques

for the combined modality treatment of spontaneous neoplasms.

Major Findings:

1) Platelets:

A. Transfusions: During the first nine months of this year 2,180 units of HL-A selected platelets were collected and transfused into 28 alloimmunized recipients. Ninety other patients were supported with random donor platelets (4,600 units) purchased from a commercial blood bank source. Development of the platelet procurement contract during the spring of 1974, will permit the transfusion of the remaining patients with platelets selected for histocompatibility variables. During this same time, 120 units of platelets were collected and shipped to six patients not cared for at the NIH. 484 units of platelets were harvested in other blood centers and sent to the NIH for Cancer Institute patients. 276 units of platelets were distributed to blood banks in the Washington vicinity for non-NIH patients.

B. Histocompatibility: The file of histocompatible (HL-A typed) donors has been enlarged and now approximates 7,000 donors. Approximately 50 new individuals are typed and added to the file each week. The initial steps for the development of a "national pool" of HL-A typed blood donors have been made in collaboration with the National Heart and Lung Institute. Initially five contributing centers (who have had their HL-A typing compared and standardized to the NIH typing) will enter names of available blood donors. If this national pool proves feasible and donor centers can exchange platelets without difficulty, the use of the national pool will be expanded to other interested cancer centers. Continued utilization of donors typed for HL-A antigens has revealed that approximately one-third of donors selected solely on the basis of HL-A type fail to provide compatible transfusion responses in alloimmunized recipients. It was recognized during this past year that reprocessing of platelet concentrates with removal of contaminating leukocytes (lymphocytes and monocytes) corrects the incompatible responses. Recipients failing to respond except to reprocessed platelet concentrates were shown to have leukoagglutinating antibody against donor leukocytes.

2) Granulocytes:

A. Transfusions: During the past nine months a controlled randomized study was begun to evaluate the efficacy of granulocyte transfusions. In this study patients are randomized between two available leukocyte collection techniques and a no transfusion group. Prior to randomization all patients are stratified as to good and poor prognosis. To date, twelve patients have been randomized for 13 episodes of gram negative bacterial septicemia. Twenty-two patients were not randomized because of inavailability of a cell separator. To date the results of this study remain too preliminary to draw a conclusion regarding the efficacy of granulocyte transfusions. A separate study examining the use of granulocytes for localized bacterial infection has demonstrated complete clearance of infection in three patients randomized to receive granulocyte transfusions.

Study of serum from patients transfused with granulocytes has identified 15 new granulocyte antigen specificities. These antigen specifications have been analysed with regard to similarity to HL-A antigens and have been shown to be separate and distinct from the HL-A antigen system. Granulocyte antigens will now become important in further identifying histocompatibility in donor-recipient pairs for granulocyte transfusion. These initial results indicate that granulocyte donors will have to be selected on the basis of granulocyte type as well as HL-A antigen matching.

B. Preclinical Studies: Using the Pseudomonas pneumonia canine model a study of clinical pneumonias was developed in irradiated dogs. Studies evaluating cells collected with the Continuous Flow Blood Cell Separator have revealed signigicant benefit in animals receiving antibiotics plus granulocytes for the treatment of Pseudomonas pneumonia (4/7 survivors) as contrasted to those animals receiving antibiotic therapy alone (0/7 survivors). Studies were initiated which examine the utility of "leukopak" processed granulocytes. These cells transfused in similar doses provide antibacterial effectiveness. It is of interest that the kinetics of the cells following transfusion were similar to those observed in human studies in which lower post-transfusion increments were regularly measured. In neither of the pneumonia models was pulmonary toxicity recognized. These results are in contrast to clinical experience where it has been suspected that patients with clinical pneumonia should not be given granulocyte transfusions for fear of causing cardiopulmonary compromise. Alloimmunization may play a greater role in clinical toxicity. Future studies will examine the role of passive antibody immunization to Pseudomonas organisms, dose and schedule of granulcytes for transfusion, and effectiveness of granulocyte transfusions in the face of alloimmunization to donor blood products.

3) Bone Marrow Transplantation:

A. Clinical: Clinical bone marrow transplantation program has continued at a low level of effort. A total of nineteen patients have been transplanted since 1969. A single patient with acute lymphocytic leukemia was transplanted from his identical twin brother using cyclophosphamide and total body irradiation for preparation. He remains in clinical remission from leukemia for 3+ months and is being given post-transplant immunotherapy with BCG-RAJI Cell immunizations. A second child with acute lymphocytic leukemia remains in clinical remission for 2+ months following transplantation of HL-A and MLC identical bone marrow. This patient demonstrated no evidence of graft versus host disease following transplantation. A single patient with chronic myelogenous leukemia was transplanted on two occasions, the first following combination chemotherapy (BACT-BCNU, cytosine arabinoside, cyclophosphamide, and 6-thioguanine); and the second following cyclophosphamide and total body gamma irradiation (TBI). This patient failed to clear his primary tumor following combination chemotherapy preparation but was subsequently successfully engrafted following the cytoxan-TBI regimen. Following a moderately severe course of graft versus host disease for which he was successfully treated with anti-lymphocyte serum, he developed an interstitial pneumonia and succumbed 110 days post-transplant with cytomegalo-virus infection. Two patients with acute lymphocytic leukemia died during the first month post-transplant of radiation hepatitis following cyclophosphamide and total body irradiation. Subsequent patients were given lower doses of total body irradiation and recovered without evidence of hepatotoxicity. A patient with acute myelogenous leukemia was transplanted from an HL-A matched sister of different major red blood cell group. In preparation for the transplant the patient was given two plasma exchange transfusions followed by intravenous infusions of a soluble B substance (Witebsky) and transfusions of incompatible red blood cells. Following these manipulations the patient was given combination chemotherapy (BACT) for immunosuppression prior to transplantation. Three days following the marrow infusion, the patient expired from cytoxan related cardiomyopathy. It is felt that this patient developed unusual toxicity from high dose cytoxan related to the altered pharmacokinetics of cytoxan metabolism which was affected by the prior plasma exchange transfusions and possible renal compromise related to incompatible red blood cell transfusions. Currently a patient transplanted 2 1/2 years ago for acute myelogenous leukemia refractory to all chemotherapy remains in clinical remission on no maintenance therapy. During the past year he has required no treatment and remains clinically well.

Antilymphocyte serum (Upjohn) has been used to treat graft versus host disease (GVHD) in 4 patients. All patients were documented to have clinical and histopathologic improvement.

B. Preclinical Canine Transplantation Studies: Experiments in the following areas were completed with the following conclusions.

1. L-aspariginase was used to reduce the incidence of graft rejection (0/13) versus TBI alone (6/15). This immunosuppressive

agent was also found to be useful for enhancing graft take with usually insufficient marrow doses.

2. Methotrexate was documented to be an effective agent for modifying GVHD (3/6) but was associated with severe hematopoietic toxicity as evidenced by failure of engraftment (4/15) or rejection (5/11) as contrasted to control animals (1/19 failures and 1/18 rejections). A single histoincompatible bone marrow persists engrafed at 165+ days.

3. Antilymphocyte serum (ALS) was used to treat animals systemically using various pre and post-transplant schedules. Pre-transplant ALS produced severe toxocity as evidenced by failure of engraftment in 5 of 9 animals given autologous bone marrow and 4 of 4 allografts. *In vitro* incubation of bone marrow with ALS absorbed to remove stem cell toxicity at a dose which suppressed mixed leukocyte culture reactivity was begun. No animal was successfully engrafted which may indicate eradication of a "permissive T-cell population" from the bone marrow which may be necessary for alloengraftment. Autologous bone marrow processed in a similar manner successfully reconstituted hematopoiesis indicating that stem cell toxicity may have been successfully removed by purification of ALS.

4. Thirty-two dogs with spontaneous lymphosarcoma were admitted (15 died on admission or during within 10 days), staged, and treated with combination chemotherapy (CLOP). Following successful remission induction autologous bone marrow transplantation (ABM) was performed following TBI (1000 rads). 4 of 8 dogs given ABM-TBI remain free of disease 120+ (50+ - 185+) days as contrasted to 30 days for control animals receiving CLOP to remission and left unmaintained. Immune studies on tumor animals and 4 normal dogs treated with this protocol reveal pronounced and prolonged immunoincompetence following combined modality treatment even though all hematologic parameters have returned to normal.

5. Two dogs with cyclic neutropenia (gray collie) were successfully reconstituted to normal hematopoiesis following transplantation of DL-A/MLC matched bone marrow, indicating that non-malignant human disorders of hematopoiesis may be correctable with bone marrow transplantation.

4) Studies of Histocompatibility and Tumor Immunity:

A. Surface Differentiation Markers and Functional Characteristics of Human Peripheral Blood Lymphocytes: It was observed that human lymphocytes form spontaneous rosettes with rhesus monkey red cells.

Detailed investigations demonstrated that this feature was a property of "T" cells only. The lymphocyte receptor responsible for rhesus monkey erythrocyte adherance was demonstrated to be different for the "T" lymphocyte receptor for sheep erythrocytes. The rosette receptor for sheep erythrocytes was generally considered to be "T" cell specific; however, it could be demonstrated on peripheral blood B lymphocytes as well after neuraminadase treatment. This finding has bearing on the clinical studies now being performed which utilize E rosetting techniques to identify the origin of neoplastic cells "thymus-derived or independent". A method for effective separation of human T and B cells was developed utilizing E-rosette sedimentation techniques. This permitted the separation of T and B cells and the study of these separate populations in lymphocyte proliferation in vitro. These investigations demonstrated that T-cells proliferate in vitro upon simulation with phytomitogens (phytohemagluttinin, concanaualin A, and pokeweed mitogen) and allogeneic cells, whereas B-cells do not. In the presence of T-cells (irradiated or non-irradiated) a B-cell proliferation could be observed to phytomitogens but not to allogeneic lymphocytes. Thus mitogen-induced B-cell proliferation appears to be T-cell mediated. Monocytes were not required for lymphocyte stimulation by phytomitogens although lymphocyte proliferation is greatly enhanced in their presence. In contrast lymphocyte stimulation in the mixed leukocyte culture assay was demonstrated to be monocyte dependent. Using an in vitro chimeric system with T and B cell sex chromosome markers, T but not B cell proliferation in the mixed leukocyte culture could be directly demonstrated. Stimulatory capacity in mixed leukocyte culture could be attributed mainly to B cell bound alloantigens. Kinetics of PHA-induced lymphocyte proliferation in vitro were investigated. It could be shown that an increasing proliferative response to increasing doses of PHA was due to the earlier proliferation of a greater number of responding cells; cell cycle kinetics were independent of the strength of stimulation.

B. Studies Utilizing Direct Cytotoxicity and for the Detection of Allo-Immunization and Immunity to Tumor Associted Antigens: A method for sensitizing larger numbers of normal peripheral blood lymphocytes to histocompatibility antigens in vitro was developed. Studies are in progress to evaluate the specificity of sensitization, and to adapt the system for the possible production of lymphocytes which are cytotoxic to autologous or allogeneic tumor cells.

Studies of lymphocyte-mediated (direct) cytotoxicity (LMC) were carried out in several areas. Selected aplastic and leukemic patients receiving prolonged platelet transfusion support from HL-A matched donors were found to have substantial LMC against donor lymphocyte and granulocyte targets, even in the absence of detectable HL-A antigen mismatch. Compatible platelet transfusion increments were seen in spite of LMC. Leukemic patients in remission, and normal volunteers were examined for LMC against the human lymphoblastoid cell line, RAJI. Leukemics, both prior to and post-immunization with RAJI cells, showed positive LMC reactions as did all normals. The significance of cytoxicity against the RAJI line in normal volunteers is uncertain, but studies are underway to examine the hypothesis that LMC against RAJI may be one in vitro measure of normal T-cell function. Various sera (normal, leukemic remission and relapse) will be examined for capacity to block the "normal" LMC against RAJI. Leukemic patients were tested for LMC against autologous tumor following bone marrow transplantation. Two patients developed positive LMC which was abolished in one when administration of antilymphocyte globulin was begun for treatment of graft versus host disease.

In preliminary work to establish the feasibility of using bone marrow mononuclear cells as targets for LMC it was found that normal individuals exhibit low levels of cytotoxicity against autologous or allogeneic bone marrow in the absence of detectable LMC against peripheral targets (lymphs or polys) from the bone marrow donor. The significance of this finding is unknown but may be related to the previously mentioned cytotoxicity of normals against RAJI cells (i.e. immature marrow cells may express fetal antigens recognized as "foreign").

In conjunction with the canine spontaneous lymphoma program, untreated dogs were tested for presence of LMC against autologous tumor. Six of eight tested had significant activity. New isotopic labels for use with canine tumor cell targets are being sought in an effort to reduce the high spontaneous (background) release which has prevented uniformly good results with the ^{51}Cr isotope now in use.

Proposed Course of Project:

1) Continued evaluation of improved procurement and transfusion techniques for platelets and granulocytes. Of particular interest is the evaluation of donor selection using histocompatibility criteria and the relative effectiveness of leukocytes collected using various techniques.

2) Continued evaluation of granulocyte antigen typing in prospective donors for leukocyte transfusions.

3) Continuation of bone marrow transplantation studies emphasizing autologous and syngeneic bone marrow following preparation with chemoradiotherapy. Marrow transplantation studies for the most part will be done in collaboration with other clinical areas of NCI and other institutes.

4) Preclinical studies will be concerned with: Granulocyte transfusions into alloimmunized recipients; combined modality therapy of spontaneous canine lymphosarcoma; use of antilymphocyte serum as an immunosuppressant in conjunction with bone marrow transplantation.

5) Histocompatibility studies will continue to examine the relationship between serologic determinants (HL-A) and LD determinants (MLC) with assays measuring alloimmunization (LMC and lymphocyte dependent antibodies).

Honors and Awards:

Invited Lectures:

Malcolm I. Bull, M.D.

1. Efficacy of Platelet Transfusions from HL-A Compatible Unrelated Donors to Alloimmunized Patients. American Federation for Clinical Research, Atlantic City, New Jersey. May, 1973.
2. Spontaneous Canine Lymphoma-Combined Modality Therapy. American Association of Cancer Research, Houston, Texas. March, 1974.
3. Canine Bone Marrow, Transplantation-Alternative Models with Analysis of Factors Influencing Engraftment. Experimental Hematology Society, Houston, Texas. March, 1974.

Julian A. Decter, M.D.

1. Current Status of Clinical Bone Marrow Transplantation. Medical Grand Rounds, New Jersey College of Medicine. May, 1974.

Roger H. Herzig, M.D.

1. Prolonged Granulocytopenia from Incompatible Platelet Transfusions. American Federation for Clinical Research, Atlantic City, New Jersey. May, 1973.
2. Principles of Hematologic Supportive Care. Washington Hospital Center, Washington, D.C. December, 1973.
3. Use of Platelets and Granulocytes in the Management of Cancer Patients. Hematology Rounds, Georgetown University School of Medicine. March, 1974.
4. Correction of Poor Platelet Transfusion Response from Donors Matched for HL-A Antigens. American Federation for Clinical Research, Atlantic City, New Jersey. May, 1974.

Geoffrey P. Herzig, M.D.

1. Use of Leukocyte Transfusions for the Treatment of Bacterial Infections. Hematology Grand Rounds, Barnes Hospital, St. Louis, Missouri. September, 1973.

Gerhard R.F. Krueger, M.D.

1. Gastrointestinal Lesions in Chronic Graft-Versus-Host Disease and Their Relation to Infection and Sepsis. International Society for Experimental Hematology. Paris. June, 1973.

Hans-Peter Lohrmann, M.D.

1) Efficacy of Platelet Transfusions from HL-A Compatible Unrelated Donors to Alloimmunized Patients. Experimental Hematology Society. Paris. June, 1973.

Robert G. Graw, Jr., M.D.

1) Granulocyte Transfusion Therapy - A Review. American National Red Cross Directors Symposium, Washington, D.C. March, 1973.

2) Clinical Bone Marrow Transplantation - A Status Report. Medical Grand Rounds, Sloan Kettering Memorial Hospital, New York. February, 1974.

3) Compatible Platelet Transfusions - In Vitro Assays and Clinical Response. National Heart and Lung Institute-Contractor Workshop, St. Louis, Missouri. April, 1974.

4) Granulocyte Transfusion Therapy - Correlation of Transfusion Response to In Vitro Assays of Alloimmunization. National Red Cross HL-A Typing Conference, St. Louis, Missouri. April, 1974.

Publications:

1) Yankee, R.A., Graff, K.S., Dowling, R., Henderson, E.S.: Selection of unrelated compatible platelet donors by lymphocyte HL-A matching. New England Jour. Med. 288: 760-764, 1973.

2) Graw, R.G., Jr.: Bone marrow transplantation for the therapy of aplastic anemia. In Proceedings of the 29th Hahnemann International Symposium - "Drugs and Hematological Reactions." N.B. Dimitrov & J.J. Nodine (Eds.), New York, Grune & Stratton, Inc., 1973, pp. 343-356.

3) Hasegawa, T., Graw, R.G., Jr., Terasaki, P.I.: Micrograngulocyte cytotoxicity test. Transplantation 15: 492-499, 1973.

4) Henderson, E.S., Graw, R.G., Jr., Anderson, R.M., Yankee, R.A., Opelz, G., Lalezari, P., and Meryman, H.P.: Obstacles to routine transfusion support of myelosuppressed patients. Cancer Chemo. 34: 51-59, 1973.

5) Lohrmann, H-P., Bull, M, Decter, J., Yankee, R.A., and Graw, R.G., Jr.: Efficacy of platelet transfusions from HL-A compatible alloimmunized. Ann. of Intern. Med. 80: 9-14, 1974.

6) Dale, D.C., Graw, R.G., Jr.: Transplantation of allogeneic bone marrow in canine cyclic neutropenia. Science 183: 83-84, 1974.

7) Herzig, R.H., Stout, F.G., Herzig, G.P., Decter, J.A., Bull, M., Lohrmann, H-P, Hasegawa, T., Yankee, R.A., Graw, R.G.: Correction of poor platelet

transfusion responses from donors matched for HL-A antigens. Clinical Research 22: 393A, 1974.

8) Herzig, R.H., Poplack, D.G., Yankee, R.A.: Prolonged granulocytopenia from incompatible platelet transfusions. New England Jour. Med. (in press).

9) Krueger, G.R.F., Herzig, G.P., and Graw, R.G., Jr.: Gastrointestinal lesions in chronic graft-versus-host disease and their relationship to infection and sepsis. Experimental Hematology (in press)

10) Graw, R.G., Lohrmann, H-P, Bull, M., Decter, J., Herzig, G.P., Bull, J.M., Leventhal, B.G., Yankee, R.A., Bleyer, W.A., Buja, M., McGinniss, M.H., Whang-Peng, J., Gralnick, H.R., Krueger, G.R.F., and Henderson, E.S.: Bone marrow transplantation following combination chemotherapy-immunosuppression (B.A.C.T.) in patients with acute leukemia. Transplantation Proceedings (in press).

11) Lohrmann, H-P, Bull, M., Decter, J., Yankee, R.A., and Graw, R.G., Jr.: Efficacy of platelet transfusions from HL-A compatible unrelated donors to alloimmunized patients. Experimental Hematology (in press).

12) Dale, D.C., Reynolds, H.Y., Pennington, J.E., Pitts, T.W., and Graw, R.G., Jr.: Granulocyte transfusion therapy in experimental pseudomonas pneumonia. J. Clin. Invest. (in press).

13) Lohrmann, H-P., Graw, C.M., and Graw, R.G., Jr.: Stimulated lymphocyte cultures: Responder recruitment in cell cycle kinetics. J. Exptl. Med. (in press).

14) Lohrmann, H-P., Novikovs, L., and Graw, R.G., Jr.: Cellular interactions in the proliferative response of human T and B lymphocyte phytomitogens in allogeneic lymphocytes. J. Exptl. Med. (in press).

15) Lohrmann, H-P., Novikovs, L., and Graw, R.G., Jr.: Stimulatory capacity of human T and B lymphocytes in mixed leukocyte culture. Nature (in press).

16) Lohrmann, H-P., and Novikovs, L.: Rosette formation between human T-lymphocytes and unsensitized rhesus monkey erythrocytes. Clinical Immunology and Immunopathology (in press).

17) Lohrmann, H-P., Whang-Peng, J.: Human mixed leukocyte culture: Identification of the proliferating lymphocyte subpopulation by sex chromosome markers. Journal Experimental Med. (in press).

18) Levine, A.S., Schimpff, S.C., Graw, R.G., Jr., and Young, R.C.: Hematologic malignancies and other marrow failure states: Progress in the management of complicating infections. Sem. Hematology (in press).

19) Yust, I., Wunderlich, J., Mann, D.L., Rogentine, G.N., Jr., Leventhal, B.G., Yankee, R., and Graw, R.G., Jr.: Human lymphocyte-dependent antibody mediated cytotoxicity against non-HL-A antigens. Nature (in press).

Serial No. NCI-6830
1. Pediatric Oncology Branch, MO
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: 1) Studies on the Integration and Genetic Organization of the Oncogenic Papovavirus, SV40, Using Adenovirus 2-SV40 Hybrid Viruses

2) Studies on Acute Infection, In Vivo Tumorigenesis, and In Vitro Transformation Induced by various DNA and RNA Tumor Viruses: Viral and Host Components as Assayed by Biochemical and Biophysical Techniques

3) Studies on the AKR Murine Leukemia Virus Genome and the Activation of RNA Tumor Viruses by Halogenated Pyrimidines

4) Infectious Complications of Malignancy: Natural History, Prevention, Diagnosis, and Therapy

Previous Serial Number: NCI-6830

Principle Investigator: Arthur S. Levine, M.D.

Other Investigators: Cephas Patch, Ph.D., Sisir Chattopadhyay, Ph.D., Ian Magrath, M.D., James H. Breeden, M.D., Richard Robinson, M.D., Yvonne Wewerka-Lutz, M.D., Andrew M. Lewis, Jr., M.D., Wallace P. Rowe, M.D., Douglas Lowy, M.D., Michael N. Oxman, M.D., Thomas J. Kelly, Jr., M.D., Ph.D., Sherman Weissman, M.D., Henry Westphal, Ph.D., George Khoury, M.D., Natalie Teich, Ph.D., John Bennett, M.D., Sheldon Wolff, M.D., Herbert Reynolds, M.D., Dorothy Moore, M.S., Janet M. Hauser, M.S., Pennington, J.E., M.D., Elin, R., M.D., Schnipper, L., Siegel, S.

Cooperating Units: Laboratory of Viral Diseases, NIAID; Research Division of Infectious Diseases, Boston Children's Hospital Medical Center and Harvard Medical School; Laboratory of Clinical Investigation, NIAID; Division of Hematology, Department of Medicine, Yale University; Section of Virology, Department of Microbiology, Johns Hopkins University; Laboratory of Biology of Viruses, NIAID; Laboratory of Molecular Genetics, NICHD.

Serial No. NCI-6830

Man Years:	Total:	28*
	Professionals:	18
	Other:	10

*Does not include administrative, nursing, or paramedical services.

Project Description:

Objectives:

1) To study the integration and organization of SV40 by an analysis of the varying segments of the SV40 genome contained within adenovirus-SV40 hybrid virus DNA molecules. Such studies employ biologic, biochemical, biophysical, and electron microscopic techniques to arrive at a genetic map of an animal tumor virus. These studies also are motivated by the hypothesis that hybrid viruses are a model for understanding the behavior of integrated genomes.

2) To identify and isolate that portion(s) of SV40 which relate most directly to the virus' oncogenicity, employing the previously noted adenovirus-SV40 hybrid viruses.

3) To analyze the behavior of DNA and RNA virus-transformed cells as a function of the molecular conformation of viral DNA within these cells, and as a function of events which may control viral genetic expression.

4) To analyze the behavior of permissive cells acutely infected by DNA and RNA viruses as a function of the molecular conformation of the viral nucleic acids and as a function of events which may control viral genetic expression.

5) To study the molecular mechanisms by which the human herpes viruses establish a latent state, as it relates to recurrent infection and to putative tumorigenicity. Such mechanisms will be approached with the techniques previously applied successfully to the smaller DNA tumor viruses.

6) To analyze the mechanisms by which C-type murine leukemia virus is activated by the thymidine analogs, 5-iododeoxyuridine (IUdR) and 5-bromodeoxyuridine (BUdR).

7) To evaluate the utility and liabilities, separately and together, of various procedures designed to reduce the incidence of severe infection in patients with acute leukemia and in other compromised hosts; to evolve prophylactic techniques and equipment with greater utility than presently available.

8) To improve methods by which occult infectious agents may be detected and eradicated, e.g., latent herpes viruses, L-forms of bacteria, fastidious anaerobic bacteria, mycoplasma, and fungi.

9) To evaluate the human germ-free state, as it is induced in patients with leukemia, and to learn what the influence of this state is on normal

physiological and metabolic processes, and on the metabolism of cancer chemotherapeutic agents.

10) To improve our understanding of the natural history of unusual opportunistic infections, seen primarily in compromised hosts.

Methods Employed:

Standard biochemical and radioisotopic methods have been employed for the measurement of nucleic acid and protein synthesis. Procedures for the extraction of highly purified viral and cell nucleic acids have been further improved. New techniques for hybridization of viral and cellular nucleic acids have been developed, and modifications which permit more quantitative comparisons of heterologous nucleotide sequences have been described. Viral DNA has been analyzed by equilibrium density centrifugation (neutral and alkaline), analytical ultracentrifugation, ethidium bromide banding of superhelical DNA, temperature-absorbance profiles, and electron microscopic heteroduplex mapping techniques. Methods for separation of the strands of adenovirus DNA, using polyUG and isopycnic density separation, have been developed.

The technique of hydroxyapatite chromatography has been employed to separate single- and double-stranded DNA. Viral DNA probes have been synthesized, utilyzing the endogenous reverse transcriptase activity of murine leukemia viruses. A combined enzymatic, chromatographic, and nucleic acid hybridization approach has permitted nucleotide sequencing of the SV40 RNA transcripts corresponding to specific strands and areas of SV40 DNA.

Quantitative evaluation of the efficacy of prophylactic techniques in compromised hosts is based upon comprehensive sampling of the patients and their environment for aerobic and anaerobic bacteria, fungi, mycoplasma, and viruses. The major prophylaxis undertaken is laminar air flow isolation-filtration and chronic bowel decontamination. Routine and special culture procedures are employed, as are serologic assays.

Major Findings:

1) Studies of Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses: Isolation of Additional Hybrids Which Differ in Their Simian Virus 40-Specific Biological Properties. Four new nondefective adenovirus 2 (Ad2)-simian virus 40 (SV40) hybrid viruses have been isolated. Although these viruses (designated Ad2$_+$ND$_2$, Ad2$_+$ND$_3$, Ad2$_+$ND$_4$, Ad2$_+$ND$_5$) were clonal derivatives of the same Ad2-SV40 hybrid population, they differ significantly from each other and from the previously isolated nondefective hybrid, Ad2$_+$ND$_1$, in their biological properties or in the amount of SV40-specific RNA induced during lytic infection.

Like Ad2$_+$ND$_1$, Ad2$_+$ND$_2$ and Ad2$_+$ND$_4$ pass serially in both human embryonic kidney (HEK) and primary African green monkey kidney cells. In contrast, Ad2$_+$ND$_3$ and Ad2$_+$ND$_5$ pass serially only in HEK cells. Ad2$_+$ND$_2$ is like Ad2$_+$ND$_1$

in that it induces the SV40 U antigen, but not SV40 T antigen; however, in contrast to Ad2$^+$ND$_1$, Ad2$^+$ND$_2$ also induces SV40 TSTA. Ad2$^+$ND$_4$ induces the SV40 T, U, and TSTA antigens. Ad2$^+$ND$_3$ and Ad2$^+$ND$_5$ do not induce serologically detectable SV40 antigens and are distinguished from each other on the basis of the relative quantities of SV40-specific RNA which they induce. The induction of different SV40-specific functions suggests the incorporation of different segments of SV40 DNA within the genomes of the respective hybrid viruses.

2) Characterization of the Simian Virus 40 RNA Species Induced by Five Nondefective Hybrid Viruses. We have characterized the SV40 portions of these hybrids by relating the SV40-specific RNA sequences transcribed in cells infected with each hybrid virus to those transcribed in cells infected with each of the other hybrid viruses and with SV40 itself. RNA-DNA hybridization-competition experiments indicate that the number of unique SV40 RNA sequences transcribed in infected cells is proportional to the size of the SV40 DNA segment contained within each hybrid and, in the case of the three hybrids which induce detectable SV40-specific antigens, to the number of SV40 antigens induced. Furthermore, the SV40-specific RNA sequences transcribed from any one of the hybrids are completely represented in the RNA transcribed from all other hybrids with longer SV40 segments. Thus, the SV40 DNA regions in the five hybrid viruses appear to contain some nucleotide sequences in common. The SV40-specific RNA transcribed from Ad2$^+$ND$_4$, the hybrid containing the largest SV40 segment, is qualitatively similar to the SV40-specific RNA transcribed early (i.e., prior to viral DNA replication) in SV40 lytic infection. Thus, it appears that no significant amount of late SV40 DNA is transcribed during infection by any of the five nondefective Ad2-SV40 hybrid viruses.

3) Use of Nondefective Adenovirus-Simian Virus 40 Hybrids for Mapping the Simian Virus 40 Genome. We have examined the structure of the DNA molecules from these nondefective hybrids, using electron microscope heteroduplex mapping techniques. Each hybrid was found to contain a single segment of SV40 DNA of characteristic size (7-43% of the SV40 genome) covalently inserted at a unique location in the adenovirus 2 DNA molecule. The SV40 segments of the various hybrids formed an overlapping series with a common end point. When the results of the electron microscopic study were combined with data on antigen induction, it was found that a self-consistent map could be constructed which related specific regions of the SV40 genome to the induction of specific antigens. The order of these early SV40 antigen inducing regions in the SV40 DNA segments contained in the nondefective hybrids is: U antigen, tumor specific transplantation antigen, and T antigen, with the U antigen region being nearest the common end point.

4) Strand Orientation of SV40 Transcription in Cells Infected by Non-Defective Adenovirus 2-SV40 Hybrid Viruses. RNA synthesized in cells lytically infected by five nondefective hybrid viruses was reacted with separated strands of SV40 DNA (produced with hydroxyapatite chromatographic methods). It was found that the entire segment of SV40 DNA in each hybrid is transcribed, and that the transcript includes "anti-late" sequences and early

sequences (-strand), but not late sequences (+strand). This result supports the hypothesis that when SV40 DNA is in the linear form, as opposed to the native circular form, late information cannot be transcribed. This failure of late transcription (+strand) is also found in cells transformed by SV40.

5) Template Topography in the Early Region of Simian Virus 40. The DNAs of the five nondefective adenovirus 2 (Ad2)-simian virus 40 (SV40) hybrid viruses contain overlapping segments of the early region of wild-type SV40 DNA. The complementary DNA strands of these five viruses have been separated with synthetic polyribonucleotides in isopycnic cesium chloride gradients. The relative amounts of early and late SV40 template in the DNA of each virus were determined by RNA-DNA hybridization with late lytic SV40 RNA, which contains sequences complementary to both templates. From the distribution of early and late templates in the five overlapping SV40 segments, we conclude that either the entire early region of SV40 is symmetrically transcribed in vivo, or, more probably, that the early SV40 templates are not contiguous.

6) Induction of Cellular DNA Synthesis by the Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses. Induction of cellular DNA synthesis by the nondefective adenovirus 2-simian virus 40 hybrid viruses was examined in monkey and hamster cells. It was not possible to discriminate the effects of the hybrid viruses from those of wild-type adenovirus 2.

7) Transformation of Hamster Kidney Cells by Adenovirus 2 and the Nondefective Hybrid Viruses. Nonhybrid Ad2 and five nondefective Ad2-SV40 hybrids $Ad2^{+}ND_{1}$-$Ad2^{+}ND_{5}$) failed to produce tumors when injected into newborn hamsters. However, each of these agents transformed hamster kidney cells in vitro and the transformed cell lines produced tumors when injected into suckling hamsters. Foci of cells transformed by the hybrids could not be distinguished from foci of cells transformed by nonhybrid Ad2. Histopathologically, tumors induced by the nondefective hybrid-transformed lines were of the adenovirus type with no areas of SV40 type fibrosarcoma. All of the cell lines studied contained Ad2 T antigen and Ad2 RNA. Only the B55HK, line contained the three early SV40 antigens, U, TSTA, and T. The $ND_{4}HK_{1}$ line contained SV40 T and TSTA antigens (the latter antigen being detected only after 48-49 tissue culture passages) and SV40 RNA. The $ND_{2}HK_{2}$ line contained SV40 RNA but no detectable SV40 antigens; the other hybrid transformed lines contained no SV40 RNA. The transformation of hamster cells by the nondefective Ad2-SV40 hybrids seemed to be caused by the Ad2 genome and could not be attributed to the regions of the SV40 genome contained within the hybrids. Additionally, these results indicate that interactions between the genomes of the nondefective hybrid viruses and the hamster cells they transform are complex, and could involve the site of integration, the size of the incorporated viral genome, the transcription or translation of the viral genome, or various combinations of these processes.

8) Studies of Hamster Cells Transformed by Ad2 and Nondefective Ad2-SV40 Hybrids. By reassociation kinetics, 1-2 Ad2 genome-equivalents per cell

genome are present in the Ad2- and hybrid-transformed hamster kidney lines examined thus far. The Ad2-specific RNA sequences in these transformed cells have been studied by hybridizing transformed cell RNA to separated strands of Ad2 DNA and assaying duplexes with hydroxyapatite chromatography. RNA from Ad2-transformed cell lines saturates about 5% of the H strand and 15% of the L strand of Ad2 DNA, in agreement with the results of others studying Ad2-transformed rat embryo cells. However, RNA from the hybrid-transformed cells saturates 16-56% of the H strand and 10-22% of the L strand. These differences in the extent and strand distribution of Ad2 transcription may reflect the SV40 insertion or Ad2 deletion in hybrid genomes.

9) Structure of Two Ad2-SV40 Hybrids which Contain the Entire SV40 Genome. Two defective Ad2-SV40 hybrids ($Ad2^{++}$HEY and $Ad2^{++}$LEY) have been isolated which contain the entire SV40 genome. Upon infection of cells permissive for SV40 both hybrids give rise to infectious SV40 virions, but with markedly different efficiencies. In the case of $Ad2^{++}$HEY, nearly all cells infected with a hybrid particle yield SV40 progeny, whereas in the case of $Ad2^{++}$LEY, infectious SV40 is produced in only about one in 10^4 cells infected with hybrid particles. The structures of the DNA molecules in the $Ad2^{++}$HEY and $Ad2^{++}$LEY populations were examined using electron microscope heteroduplex methods. Both populations were found to be heterogeneous. $Ad2^{++}$HEY contained three hybrids (HEY-I, HEY-II, and HEY-III) whose genomes differed only in their content of SV40 DNA (0.45 ± .02, 1.43 ± .04, and 2.39 ± .09 SV40 genomes, respectively). $Ad2^{++}$LEY contained two hybrids (LEY-I and LEY-II) which also differed only in their content of SV40 DNA (0.03 ± .01 and 1.05 ± .01 SV40 genomes, respectively). In those hybrids which contained more than one complete SV40 genome (HEY-II, HEY-III, LEY-II) the excess SV40 DNA was shown to be organizeed as a tandem repetition. These data suggest that the various hybrid genomes within each population are interconvertible by recombination events which insert or excise an SV40 genome. It is proposed that HEY-II and HEY-III yield infectious SV40 with higher efficiency than LEY-II because their SV40 DNA segments contain longer tandem repetitions; thus, the probability of an intramolecular recombination event which results in excision of an SV40 genome is greater.

10) Adenovirus 2-SV40 Hybrids which Vary in Their Efficiency in Yielding SV40: Differences in SV40 Antigen Induction, Transcription and DNA Replication. The amount and configuration of SV40 DNA in $Ad2^{++}$HEY hybrid virions greatly increases the probability that an intact SV40 genome will be excised during lytic infection. The expectation that enhanced excision will lead to increased replication of non-hybrid SV40 DNA is confirmed by further studies; these experiments also suggest that in most $Ad2^{++}$HEY-infected cells, SV40 DNA replication does not occur by the mechanism of self-propagation. The results of hybridization-competition experiments indicate that late SV40 RNA is not transcribed efficiently in $Ad2^{++}$ LEY infection, in contrast to $Ad2^{++}$ HEY infection, and only two SV40 antigens, T and U, are detectable in $Ad2^{++}$ LEY-infected cells. A third early SV40 antigens, TSTA, is detectable in $Ad2^{++}$ LEY -infected mouse cells but not hamster cells. These differences in the state and expression of integrated SV40 DNA in $Ad2^{++}$ HEY and

$Ad2^{++}$ LEY suggest possible mechanisms which underlie major differences between SV40 lytic infection and transformation, and the $Ad2^{++}$ HEY-$Ad2^{++}$ LEY system may also be a helpful model in explaining the recovery of SV40 from transformed cells by fusion with permissive cells.

11) Evidence that the AKR Murine Leukemia Virus Genome is Complete in DNA of the High Virus AKR Mouse and Incomplete in the DNA of the "Virus-Negative" NIH Mouse. The AKR mouse has a high titer of murine leukemia virus (MLV) early in life, and virus-negative cells derived from embryos of this mouse strain can be activated to yield MLV by treatment with 5-iododeoxyuridine (IdU). In contrast to this high virus strain, the NIH Swiss mouse has a low incidence of leukemia and no MLV has been isolated from this strain ("virus-negative"). We have attempted to understand this difference between AKR and NIH mice by examining the MLV-specific sequences in nucleic acids of these mice. A single-stranded viral DNA probe was synthesized *in vitro* using AKR-MLV RNA as the template. The probe contains at least 87% of the sequences present in the 70S viral RNA; most of these sequences are present in proportions similar to their content in the 70S RNA. Using this probe in nucleic acid hybridization experiments, we have shown that NIH cell DNA and AKR cell DNA differ from each other with respect to AKR MLV-specific sequences: (1) NIH cell DNA lacks some of the virus-specific sequences which are present in AKR cell DNA; and (2) there are two distinct sets of virus-specific sequences in AKR cell DNA, whereas there is only one set in NIH cell DNA. RNA from virus-negative AKR cells grown in tissue culture contains some, but not all, virus-specific RNA sequences; however, within 48 hours after initiating treatment of these cells with IdU, the complete viral genome is represented in cellular RNA.

12) AKR Murine Leukemia Virus Genome: Frequency of Sequences in DNA of High, Low and Non-Virus Yielding Mouse Strains. Studies with a single-stranded DNA probe complementary to the RNA of mouse-tropic AKR murine leukemia virus (MLV) indicate that the complete genome of the AKR-type MLV is present in the DNA of high- and low-virus-yeilding mouse strains, while DNA of non-virus-yielding strains contains only a part of the genome. Furthermore, in those strains where the genome is complete, two populations of virus-specific DNA sequences can be identified (more abundant and less abundant species) according to their rate of association with the probe. Low-virus-yielding mouse strains contain fewer copies of the less abundant species and consequently fewer complete viral genomes than do high-virus-yeilding strains. Thus, in the nine strains tested there is a good correlation between completeness of the AKR-type MLV genome in cellular DNA and the capacity of the cells to release infectious AKR-type MLV. Moreover, the number of complete viral genomes correlates with the frequency of infectious virus production by virus-positive strains.

13) Lack of Sequence Homology Between the 70S RNA of Various RNA Tumor Viruses and the DNA of Simian Virus 40 or Polyoma Virus. No significant hybridization was detected of DNA from simian virus 40 or polyoma virus, and of 70S RNA from avian myeloblastosis virus, murine leukemia virus (Rauscher), murine sarcoma virus (Kirsten), RD-114B, simian sarcoma virus-1, or

Mason-Pfizer virus.

14) Use of a Pseudomonas Aeruginosa Vaccine in Acute Leukemia and Cystic Fibrosis. A heptavalent lipopolysaccaride pseudomonas vaccine was evaluated in 22 acute leukemia patients and 12 cystic fibrosis patients during an 18 month interval at the Clinical Center of the National Institutes of Health. Thirty-two of 34 patients had an excellent serum hemagglutinating (HA) antibody response to immunization. In comparison to cystic fibrosis patients, the leukemia patients had a smaller HA antibody response, which lasted a shorter period of time, and also experienced greater toxicity from the vaccine. The mixing of adrenal corticosteroids with vaccine greatly decreased side reactions among leukemia patients without significantly inhibiting antibody production. Previous anti-neoplastic chemotherapy had little influence on antibody response in leukemia patients, with the exception of methotrexate. Subjects with leukemia had one pseudomonas infection out of 14 bacterial or fungal infections, while two pseudomonas infections occurred in a control group of 20 acute leukemia patients. Of the twelve patients with cystic fibrosis, four experienced pseudomonas infection after vaccination.

15) Protected Environments and Prophylactic Antibiotics: A Prospective Controlled Study of Their Utility in the Therapy of Acute Leukemia. To reduce the frequency of infection in acute leukemia, we employed isolation and air-filtration facilities ("protected environment") and a prophylactic regimen that included oral nonabsorbable antibiotics. Eighty-eight randomized patients received identical remission-induction chemotherapy within one of of three groups; protected environment combined with the prophylactic regimen (Group 1); oral nonabsorbable antibiotics alone (Group 2); and neither isolation nor prophylaxis (Group 3). The groups were comparable in factors that might influence the course of leukemia and susceptibility to infection. Environmental maneuvers were effective in reducing the potential inoculum of ambient micro-organisms. Patients in Group 1 had 1/2 as many severe infections as those in Groups 2 and 3. Whereas approximately 1/4 of the patients in Groups 2 and 3 died of infection while on study, none in Group 1 died for that reason. Despite fewer infections in Group 1, no intergroup differences were found in remission rate or duration. The ultimate benefits of reduced infectious morbidity in leukemia may depend on further advances in chemotherapy.

16) Doctor-Patient Relation in Severe Illness: A Seminar for Oncology Fellows. In an attempt to deal with the anxiety aroused in young physicians who must care for terminal patients, and with the often inappropriate defenses employed in response to this anxiety, we organized a practical teaching seminar that used the principles of preventive and social psychiatry. Six to 10 oncology fellows met weekly with a psychiatrist and a senior attending physician for approximately five months. The major initial defenses of the fellows were identified as anger and denial. The most severe problems discussed were the formation of dependency relations between doctors and patients, the patient's denial of illness, the phenomenon of social death,

and the physician's identification with his patient. The last problem often appears to be a productive one, since it encourages the physician to confront the basic issues of his own life. When this confrontation had been examined, most oncology fellows became increasingly effective with their patients.

17) Fungemia in Patients with Compromised Host Resistance. Seventy patients with ante-mortem fungemia were studied on the clinical services of the National Cancer Institute. The findings and subsequent course of the patients allowed these episodes of fungemia to be related to disseminated fungal infection (44 patients), transient catheter-induced fungemia (8 patients) or clinically insignificant fungemia (18 patients). Candida fungemias were associated with desseminated mycosis when the patients were immunosuppressed, leukopenic, grew candida from three or more other sites, or did not have a contaminated intravenous catheter. Such catheters were sometimes responsible for transient fungemia but also were occasionally associated with disseminated disease. Non-candida fungemias were associated with disseminated fungal infection when the patients had multiple other sites positive, grew fungus out of more than one blood culture, and had fungemias with recognized pathogens. Almost 20% of the fungemias reported were unexplained isolates of common laboratory contaminants occurring in patients who had a single positive blood culture, had no evidence of desseminated mycosis, and received no anti-fungal therapy. In contrast, 61% of patients in the study subsequently had either autopsy-proven or clinically diagnosed disseminated mycoses. Fungemia in patients with compromised host resistance, especially with lymphoreticular or hematopoietic malignancies, is frequently associated with disseminated fungal infection.

Proposed Course:

1. Studies will continue on hamster cells transformed by Ad2 and the non-defective Ad2-SV40 hybrid viruses. We are especially interested in which portions of the Ad and SV40 genomes are integrated during transformation, if not the whole genomes. Restriction enzyme fragments of Ad and SV40 will be used as probes for specific sets of viral DNA and RNA segments in these cells. Such fragments are highly labeled with ^{33}P or ^{125}I.

2. We will examine the phenomenon of Ad enhancement in primary monkey cells by SV40. Since $Ad2^{+}ND_1$ grows well in such cells, but induces only one SV40 protein (U antigen), it is likely that U antigen has the enhancing function. This function may be to initiate translation of Ad mRNA on polysomes. We shall test this hypothesis by first purifying U antigen (using SV40 mRNA in a cell-free protein synthesizing system), and then adding this protein to an Ad mRNA-polysome complex. If U antigen does initiate translation, it will be the first demonstration that there exist in eukaryotic cells translational controls of the phage-coliform type.

3. The topography of the early templates in SV40 will be pursued by comparing nuclear and polysomal SV40 mRNA binding to separated strands of SV40 DNA, to determine the true transcriptional map and the effect of post-transcriptional processing. Distinct species of early SV40 mRNA will be analyzed on agarose gels.

4. We shall analyze the viral genome in cells transformed in tissue culture which are then passaged in animals to induce tumors. Preliminary evidence indicates that portions of the integrated genome may be rearranged in this circumstance, and the fragments remaining may be necessary and sufficient to maintain transformation.

5. A study of human Adenovirus 2 in chronically infected tonsils will be continued. The goal of the work is to determine the state of Ad2 DNA and the manner in which its expression is regulated in this situation.

6. New studies on various herpes genomes will be initiated, particularly tests of integration or episomal configuration in Burkitt lymphoma, infectious mononucleosis, H. samurai lymphomas, and human cytomegalovirus infection. The possibility of using nucleic acid hybridization for clinical diagnosis of latent herpes infections (e.g. in banked blood) will be explored. Fragment mapping to determine which portions of the herpes genome are present in nonpermissive infection (e.g. Burkitt lymphoma) will be attempted.

7. Xenotropic viruses can be isolated from mouse cells, but do not replicate in mouse cells. This group of viruses will be characterized through the technique of reassociation kinetics. There also exist strains of inbred, congenic mice which vary in their expression of murine leukemia virus-specific material. We shall attempt to correlate nucleic acid

hybridization results with biologic genetic mapping in such animals.

8. Studies on laminar-air-flow isolation will resume with the opening of the new isolation-intensive care facility in 2B. Patients with histiocytic lymphomas or metastatic neuroblastomas will be randomized between 3 groups: conventional dosage therapy in a conventional ward; escalating therapy in a conventional ward; escalating therapy in the isolation facility. We shall determine if comprehensive antimicrobial prophylaxis permits more intensive treatment and a consequently enhanced survival.

9. Studies on the utility of the limulus lysate assay for endotoxin in clinical diagnosis of gram-negative infections will be completed.

10. A prospective study of infections in children with cancer will be initiated, as well as a randomized trial of various antibiotic regimes for the empiric therapy of sepsis.

11. A variety of ancillary studies will also be conducted in the 2B isolation facility, speaking to psychological, metabolic, and microbiologic consequences of human gnotobiologic trials. Extended trials of the utility of laminar-air-flow isolation and antimicrobial prophylaxis will also be conducted under contracts to be awarded to one or more oncology centers in 1974. Animal studies in support of the human gnotobiology trials are being conducted under a contract awarded to Notre Dame University.

12. Collaborative trials of anti-viral agents, e.g. Ara-A and IUDR in herpes infections of compromised hosts, will continue.

Honors and Awards, Invited Lectures

1. A.S. Levine: "Use of the Nondefective Ad2-SV40 Hybrids in Genetic Analysis of SV40." Lab. of Biochem., NCI, 6/1/73.
2. A.M. Lewis and A.S. Levine: "Transformation by the Nondefective Ad2-SV40 Hybrids." Gordon Conference on Animal Cells and Viruses, New Hampshire, 6/25/73.
3. A.S. Levine: "Template Topography of SV40." Gordon Conference on Animal Cells and Viruses, New Hampshire, 6/25/73.
4. S. Chattopadhyay: "The AKR Murine Leukemia Virus Genome." Gordon Conference on Animal Cells and Viruses, New Hampshire, 6/25/73.
5. Patch, C.T.: "The Early Region of SV40 is not Continuous." Cold Spring Harbor Tumor Virus Meeting, Cold Spring Harbor, N.Y., 8/12/73.
6. A.S. Levine: Visiting Professor at the Cold Spring Harbor Tumor Virus Workshop, Cold Spring Harbor, N.Y., 8/3/73 (Two lectures and seminars with graduate students, on hybrid viruses).
7. A.S. Levine: Medical Grand Rounds, Memorial Sloan-Kettering Cancer Center, N.Y., 10/12/73. "Experimental Approaches to Management of Infection in the Compromised Host."
8. A.S. Levine: Visiting Professor at the Universities of New Delhi, Bombay, and Benares (India), November, 1973; lecturing on "infectious complications of cancer" and "genetic analysis of DNA tumor viruses."
9. A.S. Levine: Medical Grand Rounds, Albert Einstein College of Medicine, New York, 1/25/74, "The Doctor-Patient Relation in Severe Illness."
10. A.S. Levine: "Studies on Two Defective Ad2-SV40 hybrids which Differ in their SV40 Yielding Efficiency." Lab. of Biology of Viruses, NIAID, 3/26/74/.
11. A.S. Levine: Discussant at the NIH Combined Clinical Staff Conference, 3/38/74. "Pseudomonas Infections - New Approaches to Treatment and Prevention."
12. C.T. Patch and A.S. Levine: Invited participants at the 9th Gustave Stern Symposium on Animal Viruses, New York, March, 1974.
13. A.S. Levine: "Cancer Chemotherapy-mechanisms and Complications", visiting lecturer, George Washington Medical School, Dept. of Pharmacology, 4/23/74.

14. A.S. Levine: Visiting Professor, Dept. of Medicine, Univ. of North Carolina Medical School, 4/10 - 4/12/74; lectured on "laminar air-flow" and on "defective and nondefective hybrid viruses."

15. A.S. Levine: Science Writers' Seminar, American Cancer Society, St. Augustine, 3/26/74. "Doctor-Patient Relation in Severe Illness."

16. A.S. Levine: Panelist, ASM Seminar on Environmental Microbiology in Hospitals, ASM annual meeting, Chicago, 5/12/74.

17. A.S. Levine: Panelist, Association for Gnotobiology, Annual Meeting, University of Guelph, Ontario, 6/9/74.

18. S. Chattopadhyay, D. Lowy, N. Teich, W. Rowe, and A.S. Levine: Invited paper at the 39th Cold Spring Harbor Symposium on Quantitative Biology: The Tumor Viruses, Cold Spring Harbor, N.Y., 5/30 - 6/6/74; "AKR Murine Leukemia Virus Genome - Frequency of Sequences in the DNA of high, low, and non-virus-yielding mice."

19. A.M. Lewis, J. Breeden, and A.S. Levine: Invited paper at the 39th Cold Spring Harbor Symposium on Quantitative Biology: The Tumor Viruses, Cold Spring Harbor, N.Y., 5/30 - 6/6/74; "Studies on the Viral Nucleic Acids in Hamster Cells Transformed by Ad2 and the Ad2-SV40 Hybrids."

20. A.S. Levine: Elected to Membership in the Society for Pediatric Research, May, 1974.

10. Chattopadhyay, S.K., Lowy, D.R., Teich, N.M., Levine, A.S., and Rowe, W.P.: Evidence that the AKR Murine-Leukemia-Virus Genome is Complete in DNA of the High Virus AKR Mouse and Incomplete in the DNA of the "Virus-Negative" NIH Mouse. Proc. Nat. Acad. Sci. (U.S.) 71: 167-171, 1974.

11. Drazen, E.C. and Levine, A.S.: Laminar Air-Flow Rooms: Resources Required for Clinical Trials. J. Am. Hosp. Assoc., 48, 88-93, 1974.

12. Levine, A.S., and Schimpff, S.C.: Antimicrobial Therapy in the Compromised Host (Cancer, Organ Transplantation, and Other Global or Local Defects in Host Defences). Chapter 42. In Kagan, B. (Ed.): Antimicrobial Therapy. Saunders Publishers, 1974.

13. Patch, C.T., Lewis, A.M., Jr. and Levine, A.S.: Studies of Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses. IX. Template Topography in the Early Region of Simian Virus 40. J. Virology 13: 677-689, 1974.

14. Levine, A.S., Schimpff, S.C., Graw, R.G., Jr., and Young, R.C.: Hematologic Malignancies and other Marrow Failure States: Progress in the Management of Complicating Infections. Seminars in Hematology in press (April 1974).

15. Young, R.C., Bennett, J.E., Geelhoed, G., and Levine, A.S.: Fungemia in Patients with Compromised Host Resistance. Annals of Int. Med., in press (1974).

16. Lewis, A.M., Jr., Rabson, A.S., and Levine, A.S.: Transformation of Hamster Cells by the Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses. J. Virology, in press, 1974.

17. Siegel, S.E., Patch, C.T., Lewis, A.M., Jr., and Levine, A.S.: Adenovirus 2-SV40 Hybrids which Vary in their Efficiency in Yielding SV40: Differences in SV40 Antigen Induction, Transcription, and DNA Replication. J. Virology. In press, 1974.

18. Kelly, T.J., Jr., Siegel, S.E., Lewis, A.M. Jr., and Levine, A.S.: Structure of two Adenovirus 2-SV40 Hybrids which Contain the Entire SV40 Genome. J. Mol. Biol. In press, 1974.

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20. Pennington, J.E., Reynolds, H.Y., Robinson, R., Wood, R.E. and Levine, A.S.: Immunization Studies with Pseudomonas Vaccine in Patients with Leukemia and Cystic Fibrosis. Am. J. Med., in press, 1974.

Publications

1. Lewis, A.M., Jr., Levine, A.S., Crumpacker, C., Levin, M., Samaha, R., and Henry, P.: Studies of Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses. V. Isolation of Additional Hybrids which differ in their Simian Virus 40-Specific Biological Properties. J. Virology 11: 655-664, 1973.

2. Henry, P.H., Schnipper, L.E., Samaha, R.J., Crumpacker, C.S., Lewis, A.M., Jr., and Levine, A.S.: Studies of Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses. VI. Characterization of the Deoxyribonucleic Acid from Five Nondefective Hybrid Viruses. J. Virology 11: 665-671, 1973.

3. Levine, A.S., Levin, M.J., Oxman, M.N., and Lewis, A.M., Jr.: Studies of Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses. VII. Characterization of the Simian Virus 40 Ribonucleic Acid Species Induced by Five Nondefective Hybrid Viruses. J. Virology 11: 672-681, 1973.

4. Levine, A.S., Siegel, S.E., Schreiber, A.D., Hauser, J., Preisler, H., Goldstein, I.M., Seidler, F., Simon, R., Perry, S., Bennett, J.E., and Henderson, E.S.: Protected Environments and Prophylactic Antibiotics: A Prospective Controlled Study of their Utility in the Therapy of Acute Leukemia. New Eng. J. Med. 288: 477-483, 1973.

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6. Artiss, K.L., and Levine, A.S.: Doctor-Patient Relation in Severe Illness: A Seminar for Oncology Fellows. New Eng. J. Med. 288: 1210-1214, 1973.

7. Levine, A.S.: Germ-Free Biology and the Patient with Malignant Disease: Clinical and Preclinical Studies. Cancer Chemotherapy Reports 4: 61-71, 1973.

8. Schnipper, L.E., Lewis, A.M., Jr., and Levine, A.S.: Induction of Cellular DNA Synthesis by the Nondefective Ad2-SV40 Hybrid Viruses. J. Virology 12: 940-943, 1973.

9. Khoury, G., Lewis, A.M., Jr., Oxman, M.N., and Levine, A.S.: Strand Orientation of SV40 Transcription in Cells Infected by the Non-defective Adenovirus 2-SV40 Hybrid Viruses. Nature New Biology 246: 202-205, 1973.

21. Levine, A.S.: The Compromised Host and Infections. Proceedings of the 14th International Congress of Pediatrics, in press, 1974.

22. Reynolds, H.Y., Levine, A.S., Wood, R.E., Zierdt, C.H., Dale, D.C., and Pennington, J.E.: Pseudomonas Infections: Persisting Problems and Current Research to Find New Therapies (Combined Clinical Staff Conference). Annals Int. Med., in press, 1974.

23. Chattopadhyay, S.K., Lowy, D.R., Teich, N.M., Levine, A.S., and Rowe, W.P.: The AKR Murine Leukemia Virus Genome in High-, Low-, and Non-virus-Yielding Mouse Strains. Thirty-Ninth Cold Spring Harbor Symposium on Quantitative Biology, in press, 1974.

24. Lewis, A.M., Jr., Breeden, J., and Levine, A.S.: Studies on the State and Expression of the Viral Genome in Hamster Cells Transformed by the Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses. Thirty-Ninth Cold Spring Harbor Symposium on Quantitative Biology, in press, 1974.

SUMMARY

OFFICE OF THE ASSOCIATE DIRECTOR

Baltimore Cancer Research Center

The past year of combined clinical and laboratory investigative programs of the Baltimore Cancer Research Center has reflected partly the enlarging role of DCT in the treatment of cancer and partly the immense amount of coordinated research effort needed for a concerted attack on cancer. Negotiations have been successfully concluded for the transfer of the BCRC to the University of Maryland Hospital complex. This will provide an unparalleled opportunity for an intramural program to integrate with a multidisciplinary university teaching hospital.

Within the Clinical Area, Phase I studies have been completed on intravenous Methyl CCNU and intrathecal thiotepa (the third drug available for the treatment of meningeal disease). Phase II studies of patients with acute non-lymphocytic leukemia using azacytidine and guanazole, in patients harboring malignant glioma using intravenous Methyl CCNU, and in patients with renal cell carcinoma evaluating thiotepa, vincristine and CCNU are showing promising results. The treatment of metastatic sarcoma with adriamycin and intravenous Methyl CCNU is being undertaken in conjunction with pharmacologic and metabolic studies of these drugs.

Phase III studies continue to evaluate acute non-lymphocytic leukemia, ALL, lymphoma, metastatic testicular carcinoma. Combined modalities treatment (surgery, radiotherapy and chemotherapy) in malignant brain tumors and Hodgkin's Disease have shown significant prolongation of disease-free survival and have developed valuable data related to these disease entities. Advances in patient care include vigorous programs in infectious disease control, cancer nursing service and cell component therapy. The Patient Care Pharmacy, whose pharmacists work as part of the patient care team, provides monitoring of drug therapy through the use of unit dose systems and computer acceptable order cards.

Studies of protected environment (Laminar Air Flow Units) with oral non-absorbable antibiotics and detailed surveillance and diagnostic microbiologic culturing have indicated ways of altering the environmental balance in favor of the patient. Significantly fewer infections and increased survival has been found in patients so treated as compared with conventional modes. The large amounts of clinical, pharmaceutical, microbiologic and therapeutic data which is developed is coordinated through the use of computer resources. A special pilot study of the problem-oriented medical records system (PROMIS) is being undertaken.

Ongoing pharmacologic evaluation of the anthracycline antibiotics (daunorubincin and adriamycin) have identified daunomycin and adriamycin reductase as a purified enzyme. In addition, several new metabolites have been identified, specific intercellular localization within the cell nucleus has been shown and the effects of hepatic function upon human pharmacology and metabolism have been delineated. _In vivo_ and _in vitro_ studies of the nitrosoureas (CCNU and Methyl CCNU) have indicated the conversion of these drugs by a mixed function oxidase preparation to a number of polar metabolites which are currently being characterized. Human pharmacologic studies show rapid degradation of these drugs with moderately rapid urinary excretion. Drug-drug interaction studies, therapeutic efficacy in model systems and analysis of these compounds at the cellular and subcellular level will continue.

The evaluation of biologic markers of malignant disease, such as muramidase, alphafetoprotein, CEA, Regan isoenzyme, and plasma and urinary polyamines are being studied in detail and data correlated with type and extent of malignant disease. Semi-automated equipment for the determination of polyamine concentrations has been developed and is being tested. Other studies include the chemical conversion of plasma prostaglandins, cellular control mechanisms effecting cell growth and differentiation, and the physiologic role of poly amines in regulating cell metabolism. The interaction of polyamines and RNA synthesis and enzymatic degradation have shown marked increase in the presence of the polyamine spermidine. In addition, a human plasma ribonuclease capable of binding polyamines has been identified in patients with multiple myeloma.

Immunologic research has concentrated on systems which detect host response to tumor specific antigens, as well as methods of amplifying this response brought about by polyadenylic-polyuridylic acid (poly-AU). A toxicity-free increased survival of AKR mice has been demonstrated with this substance. The active role which complement plays in mediating tumor cell lysis is being investigated and complement concentrations have been shown to decrease as tumor burden increases. In addition, the host response in vitro to viral particles utilizing the lymphocyte-blastogenesis system, is being pursued. Studies of immune complex disease are continuing.

The Baltimore Cancer Research Center's integrated programs of clinical and laboratory investigation continue to provide a maximal therapeutic and investigative effort in the control of certain cancers. Continued participation within the Baltimore medical and scientific and academic community have provided an excellent source of patient referral and scientific enrichment. With a gradual shift of the program toward less well-controlled solid tumors while maintaining a base of responsive hematologic malignancies, new approaches to combined modalities therapy will be explored. The association with the University of Maryland Medical School and Hospital and their preclinical, clinical and postgraduate teaching programs will provide a rare resource for these investigations.

SUMMARY REPORT
Clinical Branch, Baltimore Cancer Research Center, DCT, NCI

July 1, 1973 through June 30, 1974

Progress in many areas has occurred during this fiscal year. Phase I study of I.V. methyl CCNU has been completed and the results reported to the scientific community. Phase I study of intrathecal thio-TEPA has been completed and phase II trials in meningeal leukemia have begun. Phase II trials of azacytidine and guanazole in adult acute nonlymphocytic leukemia are nearing completion with the result that azacytidine has been identified as a highly active agent in this disease. Phase II study of I.V. methyl CCNU in brain tumors has been completed and this agent has been found to have significant activity against this tumor. Phase II evaluation of thio-TEPA, vincristine, and CCNU in renal cell carcinoma has been initiated. Phase II studies of I.V. methyl CCNU and other agents in metastatic sarcoma are well underway. Phase III studies in acute nonlymphocytic leukemia, adult acute lymphocytic leukemia, disseminated Hodgkin's disease and non-Hodgkin's lymphoma, and metastatic testicular carcinoma continue to yield valuable data. Phase III study of methyl CCNU and epipodophyllotoxin (PTG) in brain tumors are in progress.

Combined modality therapy studies (Phase IV) in Hodgkin's disease utilizing surgery, radiation therapy, and combination chemotherapy have resulted in significant prolongation of disease-free survival.

Participation in oncology programs at the two medical schools in Baltimore and in other hospitals around the community has provided a source of patient material for our studies and has enabled us to bring to the practicing and academic physician in the community the impact of the therapeutic advances resulting from the DCT program.

Protected environment (Laminar Air Flow) studies have been enhanced by detailed microbiologic surveillance and diagnostic support. This effort has led to an understanding of the microbiologic environmental impact upon the patient and has indicated ways of altering the environmental balance in favor of the patient. Specific studies have examined *pseudomonas* infection, colonization of the environment and improved culture taking and retrieval systems. Viral diagnostic surveillance is being studied in detail for its efficacy with respect to the management of the immune suppressed host.

Results to date in this area indicate that adult acute nonlymphocytic leukemia patients treated in a protected environment have significantly fewer infections during treatment and survive significantly longer than those treated in a conventional environment.

Biologic markers of malignant disease such as muramidase, alpha fetoprotein, carcinoembryonic antigen, Regan isoenzyme, and plasma and urinary polyamines have been studied in detail and the data correlated with the type and extent of malignant disease.

This mass of data has been prepared for analysis and analyzed by a pilot computer-organized problem-oriented medical system. Doctors, nurses, pharmacists, and other related personnel that take part in patient care and clinical research contribute their input to the record through this computerized system.

Plans for the next fiscal year include immune adjuvant studies in bronchogenic carcinoma, acute leukemia, and malignant melanoma. Phase II and III chemotherapy-immunotherapy studies in genito-urinary cancer are also planned. Phase I and II trials of chemotherapeutic agents administered intrathecally will be pursued.

Serial No.: NCI-6027
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Microbiological Services for Clinical and Research Studies for Cancer Patients at BCRC and at NIH, Clinical Center by Contracted Laboratory Facilities.

Previous Serial Number: Same

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Marcia R. Moody, M.S., Robert C. Good, Ph.D., Robert W. Chambers, M.D., Norman R. Friedman, B.S., Dolores M. Kenton, B.S., Geetha S. Sebastian, M.T., Arthur S. Levine, M.D., Cephus Patch, Ph.D., Janet Hauser, B.S., Martin H. Cohen, M.D.

Cooperating Units: Medical Service, NCI, BCRC. Leukemia Service, National Cancer Institute, NIH, Bethesda, Maryland; Hazleton Laboratories, Vienna, Virginia; Viral Diagnostics Laboratory, Georgetown University Hospital, Georgetown University, Washington, D.C. and Medical Oncology Branch, Veterans's Administration Hospital, Washington, D.C.

Man Years (July 1, 1973 - June 30, 1974)

Total: 3
Professional: 3
Other: 0

Project Description:

Objectives:

1. To supply viral and bacterial diagnostic services of a high quality for the study of actual and potential infection of cancer patients and of a versatility necessary to support cancer chemotherapy research programs.

2. To carry out studies of the environment as well as surveillance cultures of the patient flora in an effort to provide microbiologically controlled Laminar Air Flow Rooms and information on the relationship of organisms found in the environment to those harbored by the patient.

3. To investigate the behavior of viral agents in immunosuppressed patients whose bacterial flora has been suppressed by oral, non-absorbable antibiotics.

Methods Employed:

Samples for bacterial, viral and environmental studies are collected in transport media and/or on primary culture media. They are transported daily by chauffeur driven government vehicle to the contracted bacteriological or viral diagnostic laboratories. Presumptive results and all other new data are sent twice daily by teletype or shared computer facilities. Final reports are computer generated and the data stored for research purposes. Frequent telephone conversations, site visits and constant attention to the reported information ensures high quality. Both contracted laboratories are inspected by the Center for Disease Control, Atlanta, Georgia and maintain their approval status. The microbiologic data generated are used both for patient management and as a basis for research reviews.

Major Findings:

A total of 14,731 specimens were processed during the calendar year of 1973, 8185 during the first six months and 6546 during the second six months. An average of 625 samples per month were sent from the Medical Service, NCI, NIH and routinely analyzed for aerobic, anaerobic and fungal flora. The other approximately 1,165 samples each month were from the Medical Service, BCRC, DCT, NCI. Of these, an average of 205.4 per month were blood cultures.

A total of 842 samples were received for viral cultures during calendar year 1973. Net isolation rate for these samples (taken mostly for surveillance purposes) was 4%. The total number of tests performed on the 882 sera sent for antibody titer determinations was 17,640. Total number of significant titer fluctuations was 655; 269 or 41.07% from BCRC, 309 or 47.18% from NIH and 77 or 11.76% from VA. Of the sera positive for HAA, 94.5% were from NIH.

This program continues to use outside contracted facilities for accurate and current microbiologic data for daily patient care. The Contractors have responded to indicated needs by adopting new schema for more precise speciation of gram negative rods which give negative reactions on triple sugar iron agar and by developing accurate parameters for carbenicillin disc susceptibility testing. They are presently doing the same for the new antibiotic, ticarcillin, which is in use at BCRC. An investigation is currently in progress to determine possible clinical correlation with significant fluctuations in viral antibody titers.

Significance to Bio-medical Research:

The successful operation of contracted bacteriologic and viral services for use in direct patient care provides support for the cancer patients receiving chemotherapeutic agents. It provides the clinician with the immediate information needed in care of the patient, keeps him abreast of developing resistance to antibiotics in use, warns of cross contamination, and for patients with lowered host defense, provides data concerning the bacterial flora and antibiotic susceptibility prior to development of infection. The data generated by the program also provided the base for a number of research investigations.

Proposed Course:

To continue the program along the same lines.

Honors and Awards: None

Presentations: None

Publications: None.

Serial No. NCI-6039
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: A Computerized Microbiology Reporting and Patient Information System for use by a Distantly Located Contracted Laboratory Facility.

Previous Serial Number: Same

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: John W. Gerster, William L. Robison, Richard K. C. Hsieh, Dr.P.H., Stephen C. Schimpff, M.D., Dennis George and Darius Georg.

Cooperating Units: Medical Service, BCRC; Health Services Research, U.S. Public Health Service Hospital, Baltimore, Md.; Division of Computer Research and Technology, Data Management Branch (DCRT, DMB).

Man Years (July 1, 1973 - June 30, 1974)

Total:	1/2
Professional:	5/12
Other:	1/12

Project Description:

Objectives:

1. To provide a rapid, practical means of preliminary reporting by the use of computer facilities the clinical microbiologic data generated by a distantly located laboratory facility which is utilized by clinicians for patient support.

2. To provide a means of permanently storing BCRC patient and microbiologic data for use by clinicians and microbiologists to perform prospective and retrospective studies, and summarized final reports for the patients charts.

3. To provide error detection systems and quality control for the microbiologic data generated by the contracted laboratory.

Methods Employed:

Patients' names, hospital numbers, dates, samples, and requests are entered from the hospital into data files stored on Tymshare timesharing computer system. This information is immediately available to the distant contracting laboratory which then enters laboratory results as they become available. The new data is received twice daily by the hospital for clinical use. As each report becomes final it is transferred to a separate file and batch processed at the hospital computer center at which time the final report is produced. This data is permanently stored on mass storage disc and is used as the data base for data retrieval.

Major Findings:

The patient census program (see Project Report NCI-6220) which records patient vital statistics, rooms, beds, attending physician, diagnosis, etc., is used by the Microbiology Reporting System to detect errors in hospital numbers, names, etc. It also serves as a base for epidemiologic reports of various pathogenic microorganisms and other data summaries.

The microbiology preliminary reporting system is being intensively reviewed through the combined efforts of personnel at BCRC and DCRT, DMB, NIH with the goal of DCRT, DMB assuming responsibility for the program.

The Microbiology final report generating, data storage and retrieval system continues to operate efficiently. The retrieval programs provide for retrieval by multiple parameters for use in patient care as well as for various research purposes. The data bank contains all of the patient data generated since July 1968, when the first microbiology contract was instituted and all of the surveillance culture data for both patients and environment of the Laminar Air Flow rooms.

Significance to Bio-medical Research:

The census program enables the contracted laboratory to obtain current patient information necessary to the optimal processing of specimens. This program, together with microbiologic data, allows study of the transmission of specific microorganisms within the hospital.

The microbiology preliminary reporting system provides a means of keeping preliminary information on bacterial culture results at hand for the clinician's use. It allows immediate review of all current information on cultures of an individual patient or of all of a particular species of organism present in patients or in the environment of the Laminar Air Flow rooms. The system also expedites the transfer of the data from the culture reports as they become final to magnetic tape for batch processing and storage at the hospital Computer Center.

The microbiology data storage and retrieval system generates the final report of the microbiology culture results for placement in the patient's chart, processes the data through a data screening and error detection program which produces an error report and a report of unusual results for review by the chief microbiologist, produces data summaries inclusive of any desired information, produces infection and antibiotic susceptibility reports and other data summaries as needed.

Proposed Course:

The microbiology preliminary reporting system is undergoing revision by DCRT, DMB, NIH personnel and it is presently planned to utilize their facilities for the program.

The microbiology final report generating, storage and retrieval system will continue to operate as at present.

Honors and Awards: None.

Presentations: None.

Publications:

Vermeulen, G.D., Gerster, J.W., Young, V.M. and Hsieh, R.K.C.: A computerized data storage and retrieval system for clinical microbiology. Am. J. Clin. Path. 61:209-215, 1974.

Serial No.: NCI-6219
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies directed Toward Elucidation of Cancer Patient Microbial Flora, Colonization by Specific Bacterial Genera and Infection.

I The Detection of *Pneumocystis carinii* Infections in Cancer Patients.

II An Investigation of *Klebsiella* spp. Isolated from Cancer Patients, their Serotype and Antibiograms.

III Determination of the Species and Incidence of Gram Negative Rods and other Organisms Recovered from Various Sites in Cancer Patients.

IV Studies of New or Unusual Organisms Recovered from Cancer Patients.

V A Study of *Proteus* spp. Colonization and Clinical Significance of these Species in Cancer Patients.

VI A Study of Anaerobes in Cancer Patients.

VII Investigation of Contamination of Products Used in the Care of Cancer Patients.

VIII Evaluation of Protective Environment for the Support of Cancer Patients Receiving Chemotherapy. (See NCI-6217)

IX A Study of Hospital-Acquired Bacteria and Subsequent Infection by these Organisms.

X Combined Antibiotic Therapy for the Treatment of Cancer Patients with Infection. (See NCI-6266)

Previous Serial Number: Same. Also see NCI-6273 (Part VII above).

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Marcia R. Moody, M.S., Robert C. Good, Ph.D., Norman R. Friedman, B.S., Dolores M. Kenton, B.S., Lemuel A. Moye III.

Serial No.: NCI-6219

Man Years (July 1, 1974 - June 30, 1974)

Total: 3
Professional: 2-1/2
Other: 1/2

Project Description:

Objectives:

The objective of this project is to develop information concerning the microbial flora of cancer patients, i.e., source, colonization, infection, response to infection and protection from infection. More specifically the objectives are as follows:

I To develop a diagnostic test for infection with Pneumocystis carinii to obviate the necessity of using lung biopsy or specialized techniques such as fluorescent antibody as the only diagnostic tools.

II To continue to serologically identify Klebsiella pneumoniae by means of the Quellung (capsular swelling) test to determine colonization with subsequent infection and possible sources and to look for correlation between such serological results and antibiotic resistance.

III To continue to evaluate the species and incidence of organisms recovered from various sites in cancer patients for the purpose of monitoring the risk to individual patients and detecting trends toward significant changes.

IV To continue to study new or unusual organisms that are recovered from cancer patients. To accurately determine species and their source, area of colonization and length of colonization.

V To develop a system for subspeciation of Proteus and utilize it to determine sites of colonization and significance of colonization of cancer patients by these microorganisms.

VI To initiate improved techniques for the collection and culture of fastidious anaerobes and compare the results obtained by usual routine techniques.

VII To determine the presence of bacterial contaminants in blood and blood products, food, medications, etc. that are used by or in the care of cancer patients.

VIII To evaluate protective environments such as those provided by Laminar Air Flow rooms and Med-Assist filtration systems for the protection of cancer patients at high risk of infection due to immunosuppression.

IX To continue to perform surveillance cultures on patients and their environment to determine which microorganisms are hospital acquired, source of the infecting bacteria when possible and subsequent rate of infection by the hospital-acquired organisms.

X To cooperate in determination of efficacy of combined antibiotic therapy for infected cancer patients, establish whether blood serum of treated patients effectively kill the infective organism and determine antibiotic susceptibility of the organisms.

Methods Employed:

Pneumocystis carinii antigens prepared from infected rats are being evaluated in gel diffusion tests. Slides prepared from the rat material are being utilized to compare the effectiveness of various staining techniques.

Standard biochemical tests are used to identify Klebsiella spp. recovered from patient samples and antisera are utilized in the Quellung test to determine the serotype of organisms.

Data retrieval programs for microbiologic culture results are utilized to determine the incidence of bacteria recovered from various patient samples, to detect any trends toward significant changes which would warn of impending epidemics or increasing antibiotic resistance.

Standard and extensive specialized microbiological techniques are utilized to speciate new or unusual microorganisms and patients colonized with such organisms are cultured repeatedly to determine areas and length of colonization.

A modification of the method described by Craddock-Watson to test for bacteriocin production and tests for bacteriocin, bacteriophage sensitivity and the Dienes test were utilized to devise a standardized, reproducible means of subspeciating the Proteus group for surveillance purposes. A multiple inoculation system capable of inoculating 24 producer broths simultaneously was devised to facilitate the study.

Samples were collected with swabs kept in an anaerobic atmosphere and inoculated into prereduced media by the Virginia Polytechnic Institute system of maintaining anaerobiosis. Antisera against certain common anaerobes were prepared and are being tested to attempt to obviate the necessity of using expensive gas chromatography techniques for identification purposes.

Blood, blood platelets, rinse water from the platelet phoresis apparatus, misters, vaporizers, catheter tips and needles and occasionally other items are cultured by standard microbiological procedures to detect the presence of contaminating microorganisms.

Surveillance cultures of Laminar Air Flow Rooms and the patients on the LAF protocol are performed according to standard microbiological procedures. Filtration units (Med-Assist Corporation) placed at the head of the bed (positive pressure) and foot of the bed (negative pressure) which are used to produce bacteria-free air flowing in a laminar direction are also being evaluated as a protective environment. Surveillance cultures of the room and patients are taken according to protocol and performed according to standard procedures.

Surveillance cultures are taken on all patients with leukemia on arrival at the hospital and twice weekly thereafter. Other patients are cultured on entrance into the hospital and monthly thereafter. Patients in the Intensive Care Unit are cultured one or more times each week. Diagnostic cultures are performed as needed. Cultural results are reviewed to determine which organisms are hospital acquired and which subsequently cause infection.

All patients who are placed on the triple antibiotic therapy protocol are monitored for infecting organisms. The organisms are requested from the contracting laboratory and the strain together with sera from the same date are sent to a cooperating laboratory for determination of synergistic antibiotic activity against the organisms. Also serum "cidal" activity and/or antibiotic levels of the sera are determined.

Major Findings:

It has been determined by means of gel diffusion that Pneumocystis carinii from rats share antigens with P. carinii which infect man. The Bowling, Smith and Wescott (Am. J. Med. Technol. 39:267, 1973) stain for P. carinii utilizing cresyl echt violet and napthol yellow S has proved satisfactory.

Results of Klebsiella spp. capsular typing continue to confirm the findings of the previous year. In addition it has been shown that the capsular type being carried by a patient may change one or more times during hospitalization showing that new serotypes are being acquired from the hospital environment. The data are under analysis.

Recent trends of recovery rates of various organisms from patients showed a somewhat smaller number of Pseudomonas aeruginosa being recovered and an increased number of Staphylococcus aureus. Reasons for the change are being sought. A sharp increase in carbenicillin-resistant P. aeruginosa for prevalent serotypes (30% for serotype 1) has been observed.

Of the collection of microorganisms which were difficult to speciate, one group has been identified as Alcaligenes odorans. Another group had developed a yellow pigment and has been placed in the genus Flavobacterium, but not as yet speciated. Another group which ferments only pentose is still under study.

Some 570 Proteus isolates from 166 cancer patients were placed into 33 indicator types based on their sensitivity to a set of 38 selected bacteriocin producing strains. The method developed is rapid, convenient and reliably scored; it utilizes a 24-pronged multiple-inoculation device. Some strains were refractory to typing by bacteriocin sensitivity. Such typing was compared to typing by means of bacteriocin production as tested against a set of 20 standard indicator strains, sensitivity to an array of 19 Proteus bacteriophages and the confluent-swarm test of Dienes. Clinically significant Proteus strains were most efficiently, reliably and inexpensively typed by the combination of biochemical characterization for placement into species, bacteriocin sensitivity tests and the Dienes test. P. morganii cultures can be immediately recognized by their sensitivity to an agent produced by a Pseudomonas aeruginosa strain. A practical system for storage of Proteus cultures frozen in dimethylsulfoxide was developed. Initial studies on the human ecology of Proteus spp. indicate that many individuals carry single, unique Proteus strains and that this carriage persists over long periods of time.

An evaluation of the results obtained by utilizing optimal techniques for the cultivation of anaerobes has not been made due to loss of personnel.

Approximately 25 cultures of platelets were received weekly for examination for bacterial contaminants. One Staphylococcus aureus and approximately a 3% contamination rate with Staphylococcus epidermidis or diphtheroids were revealed. Cultures of other products did not shown contamination.

For the results of the Laminar Air Flow room as a protective environment see report of NIC-6217. Evaluation of the Med-Assist filtration system shows thus far that two patients who were heavy carriers of Pseudomonas aeruginosa and Staphylococcus aureus, respectively, did not shed any of these microorganisms into the environment. Neither did any of the five patients acquire any pathogens from the environment. Bacterial counts of organisms from environmental sources were sharply reduced when careful cleaning procedures as well as the air filtration systems were activated.

Culture results of the surveillance samples taken on patients and their environment were not evaluated during this period due to lack of personnel.

Sera and microorganisms from infected patients placed on the triple antibiotic protocol are being collected, but determinations have not as yet been carried out.

Significance to Bio-medical Research:

These studies help to define the species and often subspecies of micro-organisms which colonize patients, monitor prevalence to reveal epidemics, determines increasing antibiotic resistance among infecting organisms and evaluates environments for protection of patients against infection.

Proposed Course:

To continue along lines indicated.

Honors and Awards:

None.

Presentations:

None.

Publications:

Pneumonia in acute leukemia. Sickles, E.D., Young, V.M., Green, W.H., and Wiernik, P.H. Ann. Int. Med. 79-528-534, 1973.

Detection and quantitation of bacteria in platelet products stored at ambient temperature. Buchholz, D.H., Young, V.M., Friedman, N.R., Reilly, J.A., and Mardiney, M.R., Jr. Transfusion 13:268-275, 1973.

Serial No.: NCI-6223
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on *Pseudomonas aeruginosa* in a Cancer Research Center.

I Comparison of Immunotypes from Cancer and Non-cancer Patients.

II Hemagglutinin Levels to *Pseudomonas aeruginosa* in Cancer Patients.

III Incidence of *Pseudomonas* species other than *P. aeruginosa* in Cancer Patients.

IV Relationship of *Pseudomonas aeruginosa* Immunotypes to Antibiotic Sensitivity Patterns.

V Influence of Genetic Factors on the Emergence of Carbenicillin and Gentamicin Resistant *Pseudomonas aeruginosa*.

VI The Effect of Controlled Environmental and Antibiotic Regimens on the Induction of Atypical *Pseudomonas aeruginosa* in Laminar Air Flow Room Patients.

VII The Effect of Immunosuppressive Drugs and *Pseudomonas aeruginosa* on the Potientiation of Infection in the Compromised Host.

VIII Colonization and Infection in Cancer Patients by *Pseudomonas aeruginosa* serotypes.

Previous Serial Number: Same

Principal Investigator: Marcia R. Moody, M.S.

Other Investigators: Viola Mae Young, Ph.D., W. H. Greene, M.D., Dolores M. Kenton, B.S., Myron Fisher, Ph.D.

Cooperating Units: Parke Davis and Co., Detroit, Michiqan.

Man Years (July 1, 1973 - June 30, 1974)

Total:	2
Professional:	1-1/2
Other:	1/2

Project Description:

Objectives:

Pseudomonas aeruginosa is the most frequent cause of infectious death in patients receiving chemotherapy for acute myelocytic leukemia and a frequent cause of infection in other immunosuppressed cancer patients. The main objectives of this project are to compare the distribution of Pseudomonas immunotypes recovered from cancer patients with those from non-cancer patients to determine the modes by which P. aeruginosa immunotypes develop resistance to antimicrobial agents; to establish the effects of immunosuppressive therapy on the pathogenesis of P. aeruginosa in immunologically compromised cancer patients and the patient's response to this immunogenic stimulus. Recovery of other Pseudomonas spp. is to be related to colonization and/or infection of the patients and their antibiotic susceptibilities determined.

Methods Employed:

All Pseudomonas strains recovered from the patients and their environment were speciated and all P. aeruginosa strains were immunotyped using antisera supplied by M. Fisher of Parke Davis and Co. Hemagglutinin levels of patient antibodies were determined by the microtiter technique. Antimicrobial susceptibility tests were performed by the Bauer-Kirby-Sherris-Turck technique and antibiotic minimal inhibitory concentrations were established by use of an agar plate dilution technique. Pseudomonas strains other than P. aeruginosa recovered from cancer patients were speciated by a schemata derived from the systems of Stanier (minimal medium), Hugh (oxidative-fermentative, O-F reactions) and Pickett (O-F reactions utilizing buffered media). Carbenicillin and gentamicin resistant strains of P. aeruginosa were investigated for the presence of "R" factors by utilization of standard procedures. Mice and guinea pigs were inoculated with cytoxan and P. aeruginosa simultaneously; potientiation of infection and/or lethality was determined by comparison to groups of animals which received the bacterium or the immunosuppressive drug alone.

Major Findings:

This study has revealed that P. aeruginosa type 1 is the most common strain recovered from cancer and noncancer patients while types 4 and 5 are rare in cancer patients. Type 7 was the strain most often recovered from blood ($P<.01$). Types 1 and 7 caused more infections regardless of prior colonization than did the other types ($P<.01$). Conversely, type 3 rarely caused infections or colonizations. Type 1 caused more infections in leukemias and solid tumor patients and type 7 in those with leukemias and lymphomas. Urinary tract infections were most prevalent in patients with solid tumors but rare in patients with leukemias ($P<.001$) whereas bacteremias were most common in leukemias ($P<.01$). Forty three percent of the 169 environmental strains were definitely patient related and 53 (72.6%) of these were type 1.

Conversely only 8 (8.3%) of the nonpatient related strains were type 1; the most common strain recovered from this group was type 2 (38.5%). Antibiotic susceptibility testing showed the greatest variation according to type occurred with chloromycetin, neomycin, gentamicin and carbenicillin.

It is of particular interest that our prevalent types 1, 6, and 7 are developing considerable resistance to carbenicillin, 20.4, 18.6 and 30.8% respectively. Types 1 and 2 have also developed some resistance to gentamicin 16 and 7.7% having become resistant. No serotype 3 or 5 strains have yet been found resistant to carbenicillin although it is to be expected such strains will eventually be found.

Additional atypical antibiotic resistant P. aeruginosa strains have been recovered from patients within the Laminar Air Flow room. With the modification of the Laminar Air Flow protocol to include more ward patients who received nonabsorbable oral antibiotics, atypical P. aeruginosa strains which are resistant to carbenicillin and gentamicin have been recovered for the first time from patients outside the laminar air flow room.

Significance to Bio-medical Research:

These studies have led to a greater understanding of the dynamics of interaction between Pseudomonas aeruginosa in the environment and in the patient. They have made it possible to define related flora and to prepare for a potentially dangerous ecologic situation wherein resistant organisms have colonized a number of susceptible patients. They have also allowed us to predict the likelihood of severe infection in certain patient groups and to better understand the mechanism of the disease produced.

Proposed Course:

I. An updating of incidence has been competed and a publication is in preparation.

II. Two hundred and seventy-five sera have been tested for IgM and IgG levels. As the sera are being tested according to diagnosis, data are too preliminary for conclusions. Serologic studies will be continued.

III. This project will be continued as indicated.

IV. This project has been completed, but studies of P. aeruginosa susceptibility patterns are continuing as resistant strains have emerged in our patient population.

V. This project will continue along lines indicated.

VI. This investigation will be continued.

VII. Preliminary results are inconclusive and further testing is planned.

VIII. This study is completed. Publication is in preparation.

Honors and Awards:

None.

Presentations:

1. Serotypes of *Pseudomonas aeruginosa* colonizing and infecting cancer patients. V.M. Young, M.R. Moody, S.C. Schimpff and P.H. Wiernik. Presented by M.R. Moody at the Eastern Section of the American Federation of Clinical Research, Boston, Mass., 1974.

2. Esophagoscopy as a source of fatal *Pseudomonas aeruginosa* sepsis in patients with acute leukemia. W.H. Greene, M.R. Moody, R. Hartley, J. Aisner, E. Effman and P.H. Wiernik. Presented by W.H. Greene at the Eastern Section of the American Federation of Clinical Research, Boston, Mass., 1974.

3. Hospital-acquired *Pseudomonas aeruginosa*: Detection by surveillance cultures. An invited presentation by V.M. Young at a seminar entitled "Why Monitor," 75th Annual Meeting of the American Society for Microbiology, Chicago, Illinois, 1974.

4. Effect of acquisition on the incidence of *Pseudomonas aeruginosa*. An invited presentation by M.R. Moody at a seminar entitled, "The Ecology of *Pseudomonas aeruginosa* and its Role in the Colonization of Hospitalized Patients," arranged and convened by V.M. Young, 75th Annual Meeting of the American Society for Microbiology, Chicago, Illinois, 1974.

5. Serotyping of *Pseudomonas aeruginosa*. V.M. Young and M.R. Moody. An invited presentation by V.M. Young at a seminar of *Pseudomonas aeruginosa*, Walter Reed Army Institute for Research, Washington, D.C., 1973.

Publications:

1. Greene, W.H., Moody, M.R., Schimpff, S.C., Young, V.M. and Wiernik, P.H.: *Pseudomonas aeruginosa* resistant to carbenicillin and gentamicin. Epidemiologic and clinical aspects in a cancer center. Ann. Int. Med. 79:684-689, 1973.

2. Schimpff, S.C., Greene, W.H., Young, V.M. and Wiernik, P.H.: Pseudomonas septicemia: Incidence, epidemiology, prevention and therapy in patients with advanced cancer. Europ. J. Cancer 9:449-455, 1973.

3. Schimpff, S.C., Greene, W.H., Young, V.M. and Wiernik, P.H.: Pseudomonas infections in cancer patients. Contemp. Surg. 2:85-91, 1973.

4. Young, V.M. and Moody, M.R.: Serotyping of *Pseudomonas aeruginosa*. J. Inf. Dis. (accepted for publication).

Serial No. NCI-6254
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Research Activities Directed Toward Improvement of Microbial Laboratory Techniques.

I A study of the Relative Effectiveness of Different Culture Media and Techniques for the Isolation and Identification of Specific Species of Microorganisms.

II Development and Evaluation of Micromethods for Identification of Microorganisms.

III Studies of Antibiotic Susceptibilities Involving New Methodology or Newly Developed Antibacterial or Antifungal Agents.

IV Development of Radioimmunoassay Techniques for the Rapid Detection of Antigen from Infecting Microorganisms.

Previous Serial Number - Same

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Marcia R. Moody, M.S., Norman R. Friedman, B.S., Robert C. Good, Ph.D., Lemuel A. Moye III.

Cooperating Units: Hazleton Laboratories, Vienna, Virginia

Man Years (July 1, 1973 - June 30, 1974)

Total:	1/3
Professional:	3/12
Other:	1/12

Project Description:

Objectives:

To determine if the incorporation of bacitracin into culture media would enhance the recovery of Hemophilus spp. and other gram negative rods through suppression of normal flora which is abundant in the oropharyngeal area, i.e., α hemolytic streptococci. To differentiate genera bacteria into species as new methods become available. To evaluate the accuracy and efficiency of the API (Analytab Products, Inc.) microsystem for identification of the Enterobacteriaceae. To determine the susceptibility of various strains and species of yeast to pentamidine and to establish criteria for susceptibility testing for ticarcillin, a new penicillin-like antibiotic. To determine possible synergistic activity between antineoplastic drugs and antibiotics frequently used in cancer patients to eradicate infection. To develop a rapid method for detection of microbial antigen in body fluids which are normally sterile.

Methods Employed:

Chocolate agar with and without the incorporation of 12 International Units of Bacitracin were compared for the recovery rate of Hemophilus spp. Group D streptococci were differentiated into species according to biochemical procedures recommended by Facklam (Applied Microbiol. 23:1131, 1973). API strips were purchased from Analytab Products, Inc. and their instructions for the use of the strips were followed. Discs and standard preparations of ticarcillin have been obtained and regression curves against representative species and strains are being made. These will be compared with other penicillin-like drugs of known susceptibilities to determine if results against the known drugs could be substituted for the ticarcillin tests. Pentamidine, dissolved in Mueller-Hinton broth, was serially diluted in concentrations ranging from 25 micrograms to 0.05 micrograms/ml and tested against 24 fungi recovered from clinical samples. Both inhibitory and "cidal" levels were determined. Daunomycin, cytosine arabinoside, 6 thioguanine and 6-mercaptopurine are being examined for their bacteriostatic activity alone and in combination with cephalothin or gentamicin against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella pneumoniae. Standard growth curves are obtained by plotting natural logs of optical density over time and compared with growth curves obtained in the presence of drugs. Common antigen is being prepared from Escherichia coli 014 to enable preparation of tagged antisera to detect this antigen (which is common to members of the Tribe Enterobacteriaceae) in sterile body fluids.

Major Findings:

Thus far 64 sputa have been streaked to chocolate agar with and without added Bacitracin. Of 25 sputum samples from which Hemophilus spp. were isolated two were readily recovered from chocolate agar while all 25 were easily discernable on chocolate agar with Bacitracin. In addition, other gram negative rods, such as Pseudomonas aeruginosa grow more rapidly on this medium and both these organisms and yeasts not only grow luxuriantly, but are more readily recovered on these plates where the gram positive cocci are inhibited. These plates are now routinely utilized for sputum and pharyngeal cultures.

A new schema to aid in the identification of gram negative rods which produce no change in triple sugar iron agar was devised and adopted for use of the contractor. The schema is attached. A total of 186 samples from plates of primary isolation media prepared from clinical specimens were evaluated for the species of Group D streptococci present. One to four *S. faecium*, *S. durans*, *S. bovis*, and *S. equinus* were recovered; all other strains were identified as *S. faecalis*. The API system for identification of the *Enterobacteriaceae* proved to be accurate for many of the enteric bacteria, but was not useful for speciating the rather numerous organisms which are not readily classified. It is concluded that its use should be limited to the small hospital laboratory with unclassified organisms to be refined to more specialized laboratories.

Standardization of ticarcillin susceptibility tests is in progress. Pentamidine was tested for antifungal activity against 17 *Candida albicans*, 4 *Candida parapsilosis*, 2 *Cryptococcus neoformans* and 1 *Torulopsis glabrata*. Ten of 17 *C. albicans*, 2 of 4 *C. parapsilosis*, the 2 *C. neoformans* and the 1 *T. glabrata* had static levels of 6.25 μg/ml or less, but only one strain of *C. albicans* had a low cidal level (1.56 μg/ml). Although many strains are resistant, this drug deserves investigation as a possible adjunct to present antifungal therapy.

Thus far the only synergistic activity detected was between 6-mercaptopurine and gentamicin against *E. coli*. After about 6.5 hours the growth rate again began to approximate that of the control leading to the hypothesis that the effect is exerted during the logarithmic phase of growth. These studies are in progress.

Common antigen for use in tagged antibody preparation to detect antigen by RAI techniques is in preparation.

Significance to Biomedical Research:

Optimal bacterial culture techniques are essential to determining the bacterial flora of the patient and his environment, to recovering the causative agent of infections and to assessing the optimal antibiotic treatment.

Proposed Course:

To continue along lines indicated.

Honors and Awards:

None

Presentations:

Were invited to present data on standardization of carbenicillin susceptibility testing before an FDA committee convened to evaluate the advisability of recommending that 100 μg carbenicillin discs represent the standard concentration for performing Bauer-Kirby procedures. Our data confirmed that many *P. aeruginosa* isolates are erroneously reported as resistant when assayed with the 50 μg disc originally recommended by the FDA.

Publications:

"Hemophilus" in Manual of Clinical Microbiology, to be published by the American Society for Microbiology. Chapter written for new edition, by invitation.

Serial No.: NCI-6217
1. BCRC
2. Medical Oncology Section
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Prevention of Infections in Patients with Cancer

Previous Serial Number: Same

Principal Investigator: Stephen C. Schimpff, M.D.

Other Investigators: Viola M. Young, Ph.D., William H. Greene, M.D., Clarence L. Fortner, M.S., Lois Jepsen, R.N., Peter H. Wiernik, M.D., Nancy Cusack, Norman Friedman, B.S., Marcia Moody, M.S.

Cooperating Units: Research Microbiology Section, BCRC; Center for Disease Control, Atlanta, Georgia; Hazleton Laboratories, Vienna, Virginia

Man Years

Total: 7
Professional: 4 1/2
Other: 2 1/2

Project Description:

Objectives:

Infection is a major cause of serious morbidity and the major cause of death at this Center. Patients with acute leukemia tend to become infected with hospital-acquired organisms such as Pseudomonas aeruginosa; those with mycosis fungoides are susceptible to Staphylococcus aureus acquisition and infection, and patients with lymphoma are prone to develop Herpes zoster. The following programs have been designed to evaluate methods for reducing infectious complications among these patients with the intent of improving the quality of survival and increasing the chance for complete tumor remission.

A) Basic Infection Prevention Measures - Based on previous investigations, the following procedures have become standardized: 1) urinary catheters, indwelling intravenous catheters, and hyperalimentation are avoided except under extenuating circumstances. 2) All intravenous bottles and tubing are replaced daily; butterfly needles are changed every other day. 3) Staff members apply a quaternary ammonium lotion to hands on a routine basis. 4) Disposable sitz baths are utilized. 5) Dental service examines selected patients (ANLL, ALL) on admission.

Objectives (continued):

B) Protected Environments (laminar air flow room reverse isolation) and Microbial Flora Suppression (oral, nonabsorbable antibiotics) - Beginning July 1, 1970, patients with acute nonlymphocytic leukemia (ANLL)have been randomly allocated to one of three groups: 1) laminar air flow room plus oral, nonabsorbable antibiotics (LAF + A), 2) routine hospital care plus antibiotics, or 3) routine care alone without antibiotics. Patients are all treated equally with regard to nursing, medical, and supportive care. Each patient is seen daily and infections are recorded on a prospective, ongoing basis.

C) Prevention of *Staphylococcus aureus* Infection - Patients at this Center who become colonized with *Staphylococcus aureus* and who then become predisposed to infection (e.g., skin lesion necrosis in mycosis fungoides or granulocytopenia in acute leukemia) are the only patients who develop infection with this organism. Therefore, the colonized patient who will be at high risk of developing infection is treated prophylactically for 5 days with rifampicin and cloxacillin. Rifampicin is used to kill *S. aureus* residing intracellularly and cloxacillin is used for the major reservoirs found extracellularly. Patients who become infected with *S. aureus* are isolated to prevent patient-to-patient colonization.

D) Herpes zoster Prevention - Zoster tends to occur in specific subgroups of lymphoma patients (Project #6264) following exposure to other people with varicella-zoster infection. Therefore, patients with zoster are isolated to protect the noninfected, at-risk patients.

Major Findings:

A) Infection Prevention Procedures - The avoidance of urinary catheters has reduced urinary tract infections to less than 10% of all infections and less than 3% of all septicemias in acute nonlymphocytic leukemia patients. Elimination of hyperalimentation halved the incidence of *Candida* spp septicemia.

B) Laminar Air Flow Room Reverse Isolation and Oral, Nonabsorbable Antibiotics - Sixty-four ANLL patients were randomized to the initial investigation. Compared to the unprotected patients, patients in the laminar flow rooms receiving antibiotics had a significantly decreased incidence of total infections,bacteremias, pneumonias, rectal abscesses, urinary tract infections, and pharyngitis. Infectious deaths were reduced and the time to the first infection was delayed. The ward plus antibiotic patients who regularly ingested the antibiotic regimen had a very similar infection rate. Among those patients not tolerating the antibiotics, the infection rate approached that of the unprotected patients. The patients in the two protected groups had significantly higher remission rates and longer median survivals than the unprotected control patients.

Major Findings (cont'd):

C) Prevention of Staphylococcus aureus Infections - This project, begun only recently, has insufficient data to reach conclusions as yet.

D) Prevention of Herpes zoster - Herpes zoster continues to occur in about 30% of lymphoma patients (See Project #6264) suggesting that further new efforts are indicated to prevent this infection.

Proposed Course:

A) Basic Infection Prevention Procedures - Continue as before with increased emphasis on reducing acquisition of organisms from the hospital environment.

B) Laminar Air Flow Room and Oral, Nonabsorbable Antibiotics - Because of the excellent results achieved in the two protected groups, the group with neither isolation nor oral, nonabsorbable antibiotics has been discontinued. All ANLL patients now receive the oral, nonabsorbable antibiotics; laminar flow isolation is provided when available. A randomized study will shortly begin comparing isolation plus oral, nonabsorbable antibiotics (with or without air filtration) to routine hospital care plus antibiotics.

C) Prevention of Staphylococcus aureus Infection - Continue.

D) Prevention of Herpes zoster Infection - Evaluate new measures of controlling this infection such as zoster immune globulin and/or transfer factor.

Significance to Cancer Research:

The reduction in infections enumerated above in ANLL has led to less in-hospital morbidity, fewer infectious deaths early in the patient's course, and an improvement in complete remission rates.

Honors and Awards: None

Publications (1973-1974):

1. Schimpff SC, Greene WH, Young VM, Wiernik PH: Pseudomonas septicemia: incidence, epidemiology, prevention and therapy in patients with advanced cancer. Europ J Cancer 9:449-455, 1973.

2. Greene WH, Moody M, Schimpff S, Young VM, Wiernik PH: Pseudomonas aeruginosa resistant to carbenicillin and gentamicin. Ann Int Med 79:684-689, 1973.

Presentations (1973-1974):

1. Schimpff SC: Infection prevention in acute nonlymphocytic leukemia with consequent improved remission rates and survival duration. American

Presentations (cont'd):

Society of Hematology, 16th Annual Meeting, December 1-4, 1973, Chicago, Illinois.

2. Schimpff SC: Laminar air flow room and oral, nonabsorbable antibiotics to prevent infection in acute leukemia. Grand Rounds, Veterans Administration Hospital, Baltimore, Maryland, January, 1974.

3. Schimpff SC: Infection prevention and treatment in acute leukemia. Regional Meeting, American College of Physicians, Baltimore, Maryland, November, 1973.

Serial No.: NCI-6230
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Diagnostic Laparotomy and Splenectomy for Staging Hodgkin's Disease

Previous Serial Number: Same

Principal Investigators: Peter H. Wiernik, M.D.
Michael J. O'Connell, M.D.

Other Investigators: John Sutherland, M.D.
Harold E. Ramsey, M.D.
Linell Murphy, M.D.
Arthur Schantz, M.D.

Cooperating Units: Pathology, Tumor Surgery, and Radiology Departments, USPHS Hospital

Man Years:

Total:	1.5
Professional:	1.5
Other:	0

Project Description:

Objectives:

Accurate determination of extent of lymphomatous involvement with Hodgkin's disease is important for the individual patient's prognosis and therapy. Valid assessment of the efficacy of different therapeutic modalities used in a population with Hodgkin's disease is also dependent upon a precise localization of disease prior to therapy. The purpose of this study is to determine more accurately the extent of abdominal disease at the time of diagnosis and prior to therapy by performing diagnostic laparotomy.

Major Findings:

Analysis of over 125 patients with Hodgkin's disease who have undergone staging laparotomy and splenectomy has revealed that pre-operative assessment of the extent of abdominal involvement was incorrect in approximately 40% of the cases. Prediction of splenic involvement by Hodgkin's Disease was incorrect in 1/3 of patients, approximately 1/3 of abnormal lymphangiograms could not be confirmed at laparotomy, and a number of cases of involvement of nodes in the porta hepatis region outside the usual radiation therapy ports were dis-

Major Findings: (Cont'd)

covered. Occult hepatic involvement was discovered in some cases in whom liver function tests and physical examination were normal, and others suspected of having liver involvement were shown to have disease confined only to lymph nodes. These findings altered therapeutic approaches significantly in many patients.

It was discovered that patients presenting with supradiaphragmatic Hodgkin's disease had occult disease limited to the spleen, splenic hilum and celiac axis nodes in most cases in which abdominal tumor was documented at laparotomy. Extranodal in volvement was not seen in any of the 50 patients with supradiaphragmatic clinical stage I or II Hodgkin's disease, and most patients would have had all apparent disease adequately treated had extended field radiation therapy been administered without performing staging laparotomy. Therefore, we are currently randomizing patients who present with nodal disease above the diaphram who do not have the systemic "B" symptoms of Hodgkin's disease to undergo extended field radiation therapy on the basis of their clinical stage without performing laparotomy, or alternatively to undergo surgical staging with modification of therapy based on the documentation of abdominal Hodgkin's disease. Although several patients have been entered on this study, it is too early for evaluation at this time.

Patients who present with a suspicion of abdominal involvement undergo laparotomy prior to the initiation of therapy because of the relatively high risk of involvement of nodal sites outside standard radiation therapy ports, or extra nodal involvement, particularly of the liver.

Proposed Course:

Continue investigation.

Honors and Awards: None

Publications:

1. O'Connell, M.J., Wiernik, P.H., Sklansky, B.D., Greene, W.H., Abt, A.B., Kirschner, R.H., Ramsey, H.E., Murphy, W.L.: Staging Laparotomy in Hodgkin's Disease: Further Evidence in Support of its Clinical Utility. Amer J Med (In press).

2. Abt, A.B., Kirschner, R.H., Belliveau, R.E., O'Connell, M.J., Sklansky, B.D., Greene, W.H., Wiernik, P.H.: Hepatic Pathology Associated with Hodgkin's Disease. Cancer (In press).

3. Kirschner, R.H., O'Connell, M.J., Sutherland, J.L., Abt, A.B., Sklansky, B.D., Greene, W.H., Wiernik, P.H.: Staging Laparotomy in Hodgkin's Disease. JAMA 225:635, 1973.

4. Kirschner, R.H., Abt, A.B., O'Connell, M.J., Sklansky, D.B., Greene, W.H., Wiernik, P.H.: Vascular Invasion and Hematogenous Dissemination of Hodgkin's Disease. Cancer (In press).

Publications: (Cont'd)

5. Kirschner, R.H., Abt, A.B., O'Connell, M.J., Sklansky, D.B., Greene, W.H.: Splenic and Hepatic Granulomas in Hodgkin's Disease. Proceeding of the A.A.C.R. and A.S.C.O. March, 1974 (Abstract 277).

6. O'Connell, M., Murphy, L., Sklansky, D., Abt. A., Wiernik, P.: Technetium Sulfur Colloid Liver Scanning in Hodgkin's Disease. Clin Res (In press).

Presentations:

1. O'Connell, M.J.: A Critique of the Value of Staging Laparotomy in Hodgkin's Disease. Mayo Clinic, Medical Oncology Seminar, January 28, 1974.

2. O'Connell, M.J.: Surgical Staging of Hodgkin's Disease. University of Maryland Department of Surgery Grand Rounds, February 27, 1974.

3. Kirschner, R.H.: Prognostic Significance of Vascular Invasion in Hodgkin's Disease. National Meeting of the American Association for Cancer Research, Houston, Texas, March 28, 1974.

Serial No.: NCI-6259
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Extension of the Computerized Problem-Oriented Medical Information System (PROMIS) into the Cancer Treatment Area.

Previous Serial Number: Same.

Principal Investigator: Robert J. Esterhay, Jr., M.D.

Other Investigators: Paul Chang, M.D.
William R. Grove, Pharmacist
William L. Robinson, Research Programmer
Carole A. Rulenz, A.R.T.
Peter H. Wiernik, M.D.
Michael D. Walker, M.D.
Viola M. Young, Ph.D.
Lois Jepsen, R.N.
Clarence L. Fortner, Pharmacist

Cooperating Units: PROMIS Laboratory
Department of Medicine
University of Vermont
Burlington, Vermont 05401
Telephone: (802) 656-3946

Cooperating Investigators: Lawrence L. Weed, M.D.
Laura B. Weed, M.D.
George E. Nelson, M.D.
Jan R. Schultz, Research Associate
Stephen V. Cantrill, Research Programmer
Keith G. Morgan, Research Programmer

Man Years:

Total:	3
Professional:	3
Other:	0

Project Description:

Serial No.: NCI-6259

Objectives:

To extend the Computerized Problem-Oriented Medical Information System (PROMIS) to the cancer treatment area to allow the development of structured input ("cancer displays") for coupling learning with doing for all personnel who care for individual cancer patients entered on problem specific plans (research protocols).

Methods Employed:

1. The computer terminal being used for this purpose is a touch-sensitive cathode ray tube display device with electrostatic printer similar to those in use in Burlington, Vermont, where the computer is located. BCRC has been responsible for the installation and maintenance of this equipment and the telephone line to Burlington since February, 1972. All programming and operation of the computer system have been done at the University of Vermont.

2. The basic components of the remote terminal at the BCRC are the same as the local terminals in Burlington, Vermont: display screen, display memory, keyboard, Digiscribe, and communication link to the computer in the PROMIS Laboratory, Burlington, Vermont.

3. The BCRC staff has developed antineoplastic drug displays which have been integrated with the displays already on file in Burlington, Vermont as part of the PROMIS system. Currently over 20,000 displays have been developed by the PROMIS group.

4. Problem-specific display plans for acute non-lymphocytic leukemia have also been developed which includes a series of protocols for treating this specific problem. The protocol displays couple the user to the protocol specific information and ordering drug display sequences.

Major Findings:

The computerized displays for the logic and action of treating cancer problems are defined by the creation and sequencing of the computer display pages or frames. These displays provide an automatic connective device between the four phases of medical action of the Problem Oriented Medical Record. The problem defining sequence determines the specific plan, and it is that plan which is in part a protocol or series of protocols for treating a specific cancer problem. The protocol couples the user to the protocol specific antineoplastic drug displays which provide feedback loops to the patient's problem list and outstanding orders. It is through the use of these explicit informational and directional displays that the user can couple learning with doing while gathering information and treating patients' cancer problems.

Significance to Cancer Research:

1. The PROMIS system provides a comprehensive approach to bringing current cancer knowledge to bear directly on the individual cancer patient's problems.
2. Explicit definition by structured cancer displays of logic and action pathways serves as a well-defined point of departure for discussion, feedback loops and correction of problem-specific plans (research protocols) for logic and direct care of the cancer patient.
3. Inherent in the PROMIS system but not necessarily a direct consequence of the project is the physician's ability to communicate to all others who have immediate care of the patient. All individuals who work with patients can quickly ascertain what the problems are and what is being done for them.
4. The PROMIS system is a prototype for a general system which will include cancer displays as an integral part of the system for patient care and medical education.
5. The expected date for full-scale implementation of PROMIS cannot be stated at this time.

Proposed Course:

Continued study.

1. Maintainence of the BCRC-PROMIS communication linkage by the communications programmer at the PROMIS Laboratory, Burlington, Vermont and the communications programmer at the BCRC, Baltimore, Maryland.
2. Development by the BCRC staff of problem specific cancer display plans for leukemia, lymphoma, brain tumors and selected solid tumors.
3. Audit of BCRC's developed cancer displays by the PROMIS staff for accuracy, completeness, and correctness of clinical judgement in both the content of the displays and their sequencing.

Honors and Awards: None.

Publications: None.

Presentations:

1. Esterhay, R.J., Jr., Wiernik, P.H. and Fortner, C.L., Tenth Symposium on Biomathematics and Computer Science in the Life Sciences, March, 1973, Houston, Texas. "Antineoplastic Drug Displays for the Computerized Problem-Oriented Medical Information System (PROMIS)".

Lectures: Robert J. Esterhay, Jr., M.D.

1. All Medical and Dental Officers and Chiefs of Departments, U.S. Public Health Service Hospital, Baltimore, Maryland, August, 1972, "Problem-Oriented Medical Record".
2. Staff Meeting, Sacred Heart Hospital, Cumberland, Maryland, September, 1972, "Problem Oriented Charting".
3. Health Record Administration Program, Public Health Service, Baltimore, Maryland, October, 1972, "Computerized Problem-Oriented Medical Record".
4. Grand Rounds, Greater Baltimore Medical Center, Towson, Maryland, November, 1972, "Problem Oriented Grand Rounds".
5. Computers in Clinical Medicine, Division of Computer Research and Technology, National Institutes of Health, Bethesda, Maryland, February, 1973, "Computerized Problem-Oriented Medical Information System (PROMIS)".
6. Grand Rounds, Maryland General Hospital, Baltimore, Maryland, March, 1973, "Audit of the Problem-Oriented Medical Record".
7. Grand Rounds, The Johns Hopkins Hospital, Baltimore, Maryland, October, 1973, "Computerized Problem-Oriented Medical Record (PROMIS)".

Serial No.: NCI-6263
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Protocol for Staging and Treatment of Lymphosarcoma and Reticulum Cell Sarcoma

Previous Serial Number: Same

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: Michael J. O'Connell, M.D.

Cooperating Units: None

Man Years:

Total:	1/8
Professional:	1/8
Other:	0

Project Description:

Objectives:

1. To assess the value of surgical staging in patients with apparently localized lymphomas.
2. To determine whether adjuvant chemotherapy will improve treatment results in patients with localized lymphomas treated with radiation therapy.

Methods Employed:

Patients with malignant lymphomas which are apparently clinically localized (stage I or stage II) undergo staging laparotomy to detect occult foci of intra-abdominal tumor. Patients with negative laparotomy findings (pathological stage I or II) are then randomized to receive either A.) radiation therapy alone or B.) radiation therapy followed by 6 courses of combination therapy utilizing cytoxan, vincristine, and prednisone. Patients who are found to have at least stage III disease are treated with combination chemotherapy on a different protocol.

Major Findings:

Several patients with apparently localized supradiaphragmatic malignant lymphoma have been found at laparotomy to have occult disease below the diaphram which would not have been properly treated had local therapy been given on the basis of clinically apparent disease only. Six patients whose staging

Major Findings:

laparotomies were entirely negative were randomized to radiation therapy with or without adjuvant chemotherapy. Relapses have been seen in both study groups. Too few patients have been accrued at this time to determine the value of adjuvant chemotherapy in treating localized malignant lymphomas.

Significance to Cancer Research:

There is evidence that truly localized malignant lymphomas are radiocurable if high dose radiation is given to all sites of involvement. On the other hand, such approaches would not be expected to be effective in patients with disseminated disease. Employing staging laparotomy as an additional diagnostic test to determine the extent of disease, therefore, may allow better patient selection for attempts at curative radiation therapy. In addition, it should be determined whether the addition of adjuvant chemotherapy will significantly prolong disease free and overall survival.

Proposed Course:

Continue investigation.

Honors and Awards: None

Publications: None

Presentations: None

Serial No.: NCI 6264
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS-NCI
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Evaluation of the Types and Causes of Infectious Diseases in Cancer Patients

Previous Serial Number: Same

Principal Investigator: Stephen C. Schimpff, M.D.

Other Investigators: William H. Greene, M.D., Joseph Aisner, M.D., Michael O'Connell, M.D., John Levi, M.D., Nancy Cusack, Peter H. Wiernik, M.D.

Cooperating Units: Research Microbiology, Viola M. Young, PhD., Norman Friedman

Man Years:

Total:	2
Professional:	1 1/2
Other:	1/2

Project Description:

Objectives:

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aims of this project are to 1) catalogue and identify the infections manifest both clinically and postmortem, 2) identify the predisposing factors which have contributed to the specific type of infection, 3) evaluate new techniques for more rapid and/or accurate diagnosis of infection.

Methods Employed:

Beginning July 1, 1970, each patient with suspected infection has been seen by one of the investigators who examines the patient and reviews the chart, both initially and throughout the course of the infection. Detailed records are kept of history, examination, laboratory data, etc. Summaries of infections are prepared periodically and th data computerized for rapid retrieval. Similar details are obtained on infections detected at the time of postmortem examination.

Major Findings:

A) Overall Clinical Infections - This review of all infections occurring among all patients treated for cancer by a small number of physicians under protocol conditions on a single hospital floor over a 4-year period has provided a unique opportunity to detect differences in infection, type, and incidence among different tumor types and to better understand the predisposing factors to each class of infection.

1) Acute Nonlymphocytic leukemia - Infections are present in 25% of patients at admission. The first severe in-hospital infection tends to occur within the first 20 days of hospitalization unless preventive measures are utilized (see report #6217). Less than 2% of infections occur in the absence of granulocytopenia. Seventy per cent of deaths are due to infection. Infections are largely confined to 5 sites: lung, anorectum, pharynx, urinary tract, and skin with the first 3 sites being the origin of 70% of all bacteremias. The most frequent infecting organisms are Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli; these organisms also cause > 70% of all bacteremias. Approximately 50% of all infections are caused by organisms acquired after admission to the hospital with 84% of P. aeruginosa infections following hospital acquisition.

2) Hodgkin's disease - A series of 92 previously untreated patients who were staged with exploratory laparotomy and slpenectomy have now been observed for 1-5 years (median 2+ years) for evidence of infection. No serious bacterial infections occurred during the initial intensive combination chemotherapy of the 14 patients with stage IIIB or IV disease; none occurred during the six courses of adjuvant MOPP therapy given after radiation to patients with I-IIIA disease, and none occurred in any patient during remission. Eight severe bacterial infections occurred in 5 patients with recurrent Hodgkin's disease and prolonged, severe granulocytopenia. Two of the 10 deaths among these patients were caused by infection (P. aeruginosa). It is apparent that severe bacterial infection is distinctly uncommon among splenectomized patients with Hodgkin's disease unless profound granulocytopenia is present.

The major cause of infectious morbidity in these patients has been Herpes zoster - 22 cases among 92 patients. An analysis of possible predisposing factors has indicated that radiation followed by chemotherapy is a major factor whereas chemotherapy alone is not (36% vs. &%). Female patients (regardless of therapy) have a three-fold higher incidence than do male patients. Of lesser importance are anergy, lymphocytopenia, and circulating antibody to the V-Z virus. Females treated with both radiation and chemotherapy have a 64% incidence of Herpes zoster.

B) Pneumonia - Pneumonia represents the most common serious infection in all patients at this institution and causes more than 60% of the infectious deaths in acute leukemia. In leukemia patients the most common etiologic agents have been P. aeruginosa (17%), other gram-negative bacilli (29%), and fungi (25%). In only 60% of these pneumonias was the

Major Findings (cont'd):

responsible etiologic agent identified antemortem. Fortunately, the use of laminar air flow rooms with oral, nonabsorbable antibiotics (see report #6217) has reduced the incidence of early pneumonia but the need for improved diagnostic accuracy remains imperative. Therefore, a program of intensive evaluation of pulmonary infiltrates culminating with transtracheal selective bronchial brushing (see report #   ) has been instituted.

C) Hepatitis - Serum hepatitis (HAA positive) has become a significant cause of morbidity in patients who receive multiple blood product transfusions. Previously, approximately one to two cases of hepatitis occurred per month at the BCRC but in late 1973 a striking increase was observed with 13 documented cases over 3 months. Epidemiologic investigation indicated that 28% of ANLL patients admitted from July 1, 1973 had developed hepatitis. An additional group of patients were found to be HAA or HAB positive with no clinical signs of hepatitis. A review of all blood donors is underway to determine if these cases of hepatitis represented a point-source outbreak.

D) Rectal Abscesses - Perianal and perirectal infections rand second only to pneumonia as the most frequent site of septicemia in patients with acute leukemia. Twenty-five lesions were detected among 581 patients treated at the BCRC over a three year period. All but one occurred in the 101 patients with leukemia or myeloma. Of further significance was the observation that among patients with leukemia, those with acute monocytic leukemia had an incidence of 60%, acute myelomonocytic leukemia - 31%, but only 11% and 8% in acute myelocytic and acute lymphocytic leukemia. It is therefore particularly important to establish preventive measures for the patients with AMOL and AMML. (See # 6217 regarding the absence of these infections among patients placed on prophylactic oral, nonabsorbable antibiotics in laminar air flow rooms.)

E) Pseudomonas aeruginosa Infections - This organism has accounted for 28% of all septicemias at the BCRC in recent years. Prior to 1969, approximately 90% of pseudomonas septicemias progressed to death, usually in 2-3 days. Surveillance cultures indicate that nearly 25% of colonized patients will ultimately become infected but that colonization usually occurred subsequent to hospitalization. In ANLL, 15 of 22 colonized patients became infected (13 with septecemia).

Empiric therapy (see report # 6266) with carbenicillin and gentamicin has reduced this mortality to less than 40% and isolation plus oral, nonabsorbable antibiotics (see report # 6217) have reduced the frequency of this infection.

Pseudomonas aeruginosa is acquired from multiple hospital sources. Recent investigations have led to two previously unappreciated sources: food and esophagoscopy. Uncooked green, leafy vegetables frequently harbor pathogenic P. aeruginosa; only cooked food diets are now used for leukemia patients. Esophagoscopy is utilized to diagnose the presence and cause of

Major Findings (cont'd):

esophagitis. After pseudomonas infection developed following esophagoscopy, it was found that standard cleansing procedures will not kill P. aeruginosa. Therefore, esophagoscopies are now performed only following gas sterilization of the instruments.

Proposed Course: Continue investigation.

Significance to Cancer Research:

A definition of the incidence, etiology, and sites of clinical infection, and an evaluation of the diagnostic techniques extant, are necessary precursors to the rational use and planning of antibacterial and antifungal therapy and the institution of alternate ways by which to make the diagnosis of infection. The successful control of infection, in turn, is capable of significantly improving the response rate to current antineoplastic therapy by allowing dufficient survival for delivery of such therapy.

Publications:

1. Schimpff SC, Greene WH, Young VM, Wiernik PH: Pseudomonas septicemia: incidence, epidemiology, prevention and therapy in patients with advanced cancer. Europ J Cancer 9:449-455, 1973.

2. Sickles EA, Young VM, Greene WH, Wiernik PH: Pneumonia in acute leukemia. Ann Int Med 79:528-534, 1973.

3. Schimpff SC, Greene WH, Young VM, Wiernik PH: Pseudomonas aeruginosa - its significance in the patient with leukemia and lymphoma. J Inf Dis Iin press).

4. Levine AS, Schimpff SC, Graw R, Young RC: Management of infections in leukemia and lymphoma. Seminars in Hematology, April, 1974.

5. Levine AS, Schimpff SC: Antimicrobial therapy in the compromised host. Antimicrobial Agents, 2nd edition (in press).

6. Presant CA, Wiernik PH, Serpick AA: Factors affecting survival in nordardiosis. Amer Res Dis 108:1444, 1973.

Presentations:

1. Schimpff SC: Infections in 92 splenectomized patients with Hodgkin's disease. Annual Meeting, American College of Physicians, New York, New York, April, 1974.

Presentations (cont'd):

2. Schimpff SC: Infections in patients with Hodgkin's disease. University of Maryland, Department of Medicine Faculty Meeting, March, 1974.

3. Schimpff SC: Herpes zoster in cancer patients. Grand Rounds, University of Maryland, February, 1974.

4. Schimpff SC: Infection in acute nonlymphocytic leukemia. Acute Leukemia Teaching Session, American Society of Hematology, Chicago, Illinois, December, 1973.

5. Schimpff, SC: Pseudomonas aeruginosa - its significance to the patient with leukemia or lymphoma. Pseudomonas Symposium, Walter Reed Army Hospital, Washington, D.C., October, 1973.

6. Greene WH: Esophagoscopy as a source of fatal Pseudomonas aeruginosa spesis in patients with acute leukemia. Eastern Section, American Federation for Clinical Research, Boston, Massachusetts, January, 1974.

Serial No.: NCI-6266
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Therapy of Infection in Patients with Cancer

Previous Serial Number: Same

Principal Investigator: Stephen C. Schimpff, M.D.

Other Investigators: William H. Greene, M.D., Peter H. Wiernik, M.D.,
Clarence Fortner, M.S.

Cooperating Units: Research Microbiology, BCRC, Viola M. Young, Ph.D.,
Norman Friedman, B.S.;
Division of Infectious Diseases, University of Maryland,
Harold Standiford, M.D.;
European Organization for Research and Treatment of
Cancer

Man Years

Total:	1 1/4
Professional:	1
Other:	1/4

Project Description:

Objectives:

A) Prospective Randomized Controlled Trial of Three Bactericidal Antibiotic Combinations as Initial Empiric Antibiotic Therapy of Suspected Infection in Granulocytopenic Patients with Cancer - Infection is a frequent and serious complication in the granulocytopenic cancer patient (see Report #6217). Previous investigations at the BCRC indicated that the combination of carbenicillin and gentamicin was effective for *P. aeruginosa* sepsis but not *Klebsiella* or *Escherichia coli* bacteremia or pneumonia. This suggested that gentamicin alone was inadequate therapy for these two organisms (they are not susceptible to carbenicillin). Preliminary data also suggested that gentamicin alone was inadequate for *P. aeruginosa*. In a subsequent study, cephalothin was added to the original regimen and it appeared that the added drug may have improved response rates for *Klebsiella* and *E. coli* infections. The problems of broad spectrum antibiotic usage are formidable and include a high frequency of superinfections plus occasional renal damage. It was therefore decided to determine in an appropriate prospective, controlled trial whether gram-negative bacillary

Objectives (cont'd):

infections in granulocytopenic patients respond better to a penicillin-like drug (carbenicillin or cephalothin), an aminoglycoside (gentamicin), or the synergistic combination (carbenicillin-gentamicin for P. aeruginosa and cephalothin-gentamicin for Klebsiella or E. coli). Because of the large numbers of patient trials required, the BCRC has joined with an 18 hospital group to study this question.

B) Prospective, Randomized Controlled Trial to Test the Efficacy and Safety of Low-Dose Cytosine Arabinoside to Prevent the Progression of Herpes Zoster - Because zoster infections at this cancer center are common and because they have a significant degree of morbidity, and because there is preliminary evidence to suggest that cytosine arabinoside will halt the multiplication of varicella-zoster virus, it was decided to use cytosine arabinoside as systemic therapy of patients with early zoster in a controlled, randomized trial to determine whether this therapy would be better than placebo in halting the progression of infection.

Major Findings:

A) Prospective, Controlled Trial of Three Antibiotic Combinations - The trial, begun January 3, 1974, has been utilized for 62 BCRC patients as of April 8, 1974. No definitive trend favoring any one or two combinations has emerged at this early date.

B) Cytosine Arabinoside vs. Placebo for Early Herpes Zoster - Eighteen patients have been entered on study, nine to each protocol arm. One patient in each group has progressed to disseminated zoster (11% per group). Local zoster progression has been halted in equal numbers of both groups. This prospective controlled investigation indicates that, although free of major toxicities, the use of cytosine arabinoside was of no value in halting local progression.

Proposed Course:

A) Combination Antibiotic Therapy - Continue study as outlined.

B) Cytosine Arabinoside for Local Zoster - Continue study to determine if this agent will or will not prevent dissemination of zoster.

Significance to Cancer Research:

The improvements in recent years in the therapy of gram-negative bacillary infections has led to improved survival with significantly more patients having an opportunity to achieve a complete remission of their leukemia or other advanced malignancy.

See also reports #6275 and #6914 regarding granulocyte transfusions for non-responding infections and radiation therapy as adjunctive therapy in the

Significance to Cancer Research (cont'd):

treatment of localized lesions.

Publications (1973-1974)

See #6217.

Presentations (1973-1974)

See #6217.

Serial No.: NCI 6275
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973 - June 30, 1974

Project Title: Cell Transfusion Therapy

Previous Serial Number: Same

Principal Investigators: Charles A. Schiffer, M.D, Joseph Aisner, M.D., Peter H. Wiernik, M.D.

Other Investigators: Frances Sanel, Ph.D.

Cooperating Units: Baltimore Red Cross, Community Blood and Plasma Service, Dr. Paul Terasaki, University of California, Los Angeles, California; Dr. R. Rubin, Johns Hopkins School of Public Health, Baltimore, Maryland; Research Pathology Service, BCRC, Baltimore, Maryland

Man Years:

Total:	4½
Professional:	2
Other:	2½

Project Description:

Objectives:

Treatment of patients with cancer with aggressive chemotherapy and radiotherapy has produced an increased need for blood components to deal with the complications of bone marrow suppression. This program was designed to treat and prevent hemorrhage and infection in these patients.

Methods Employed:

I Platelet Transfusion - Platelets obtained from single units of whole blood are obtained from the Baltimore Red Cross while platelets obtained from two-unit plateletphereses are supplied by Community Blood and Plasma Services (CBPS). HL-A matched platelets for alloimmunized patients are provided by multi-unit plateletpheresis of related and unrelated donors performed at the BCRC and at CBPS. (HL-A typing is performed by Dr. P. Terasaki, Los Angeles, California.)

Methods Employed (cont'd):

HL-A matched platelets, autologous platelets from patients with leukemia in remission and pooled random donor platelets have been frozen using 5% DMSO and subsequently transfused to thrombocytopenic patients.

Random donor platelets are prepared and stored at room temperature for up to 48 hours prior to use although most platelets are transfused fresh. Post-transfusion count increments, template bleeding times and clinical evaluation have been used to assess response.

Recent publications have indicated that stored blood or platelets contain large amounts of elutable plasticizer (Di2-ethylhexylphthalate - DEHP). A pilot study was begun with Dr. R. Rubin, Johns Hopkins University School of Public Health, to measure the in vivo clearance of DEHP transfused with platelet-rich plasma. Results of these studies are not yet available.

II Granulocyte Transfusion - Granulocytes for transfusion to infected, neutropenic patients are obtained from normal donors using nylon fiber filters and continuous flow filtration leukopheresis (CFFL). In vitro studies of these cells procured included bactericidal assays, electron and light microscopy morphology, viability by dye exclusion, phagocytic studies, NBT reduction and muramidase release during adhesion. In vivo studies included radioisotopic scanning with ^{51}Cr and ^{67}Gallium, assessment of migration to skin windows and infected sites, post-transfusion count increments and overall clinical assessment of the recipients' courses.

During the course of CFFL it was noted that donors uniformly developed profound, transient neutropenia. Further investigation of this phenomenon using a simplified system was undertaken.

Major Findings:

I Platelet Transfusion - The efficacy of HL-A matched platelets for long-term support was confirmed and some patients have been supported for close to one year. Mixed results have been noted using frozen platelets. Freezing loss has been significant and on preliminary analysis does not seem to be related to freezing rate. Prostaglandin E_1 did not alter freezing loss when added during the thawing procedure. Cessation of hemorrhage and post-transfusion count increments as high as 74% of predicted have been seen following the transfusion of frozen platelets but many patients have shown no response at all. Bleeding times have not been shortened in any recipients.

II Granulocyte Transfusion - In vitro testing has shown that cells collected by CFFL function almost normally in vitro. Morphological changes, consisting of vacuolization and lysosomal disarray, as well as release of intracellular contents such as muramidase, have been noted. Dramatic clinical responses have been seen in some recipients but widespread parenchymal infection, particularly pneumonia caused by bacteria and/or fungi, has thus far

Major Findings (cont'd):

been resistant to therapy. Further experience with the collection system has confirmed that CFFL is the most efficient and least expensive means of obtaining large numbers of granulocytes for transfusion.

A series of experiments determined that a substance released during adhesion of granulocytes is responsible for the neutropenia observed during CFFL, probably by induction of margination. Further experiments are contemplated to isolate the substance presumably released by the granulocytes which cause this. Prior to labeling granulocytes for transfusion with ^{67}Ga, a series of experiments were carried out to measure the effect of exposure to the radioisotope on granulocyte function. Concentrations as high as 10 uc/ml did not affect granulocyte viability and did not impair phagocytosis of latex particles. Concentrations of 1 uc/ml did not alter bactericidal activities. Labeling index for granulocytes was low (5-10%) and there was no labeling of red blood cells.

Proposed Course:

I Platelet Transfusion - Attempts will be made to expand the pool of HL-A typed donors in order to provide matched platelets for more patients. Studies comparing the clinical efficacy of platelets stored at 22°C and 4°C are planned. Tests of platelet antibody designed at "cross matching" platelet donors and recipients will be carried out.

II Granulocyte Transfusion - *In vitro* testing of the mechanism of granulocyte adhesion to nylon fibers are planned. Granulocyte transfusion using CFFL will continue to be carried out and investigations will be aimed at improving the final cell yield and the functional integrity of the granulocytes procured.

Honors and Awards: None

Publications:

1. Schiffer CA, Buchholz DH, Aisner J, Wiernik PH: Clinical experience with transfusion of granulocytes obtained by filtration leukopheresis. Proceedings of the American Society of Hematology, December, 1973.

2. Schiffer CA, Aisner J, Wiernik PH: Transient neutropenia induced by transfusion of blood exposed to nylon fiber filters. Clinical Research, May, 1974.

3. Buchholz DH, Schiffer CA, Wiernik PH, Betts S: The use of frozen autologous platelets in the supportive care of patients with leukemia. Proceedings of the American Society of Hematology, December, 1973.

Publications (cont'd):

4. Schiffer CA, Buchholz DH, Wiernik PH: Intensive multi-unit plateletpheresis of normal donors. Transfusion (in press).

Presentations:

1. Schiffer CA, Buchholz DH, Wiernik PH: Intensive plateletpheresis of normal donors and its clinical application. Presented at the 26th Annual Meeting of the American Association of Blood Banks, November, 1973.

2. Schiffer CA, Buchholz DH, Wiernik PH: Transfusion of granulocytes obtained by filtration leukopheresis. Presented at the American Association of Cancer Research, March, 1974.

3. Buchholz DH, Schiffer CA, Wiernik PH: Description and evaluation of a low cost system for filtration leukopheresis. Presented at the 26th Annual Meeting of the American Association of Blood Banks, November, 1973.

4. Schiffer CA: Granulocyte transfusion and histocompatibility testing. Presented at the Symposium on Clinical Review of Blood Transfusion Therapy and Clotting Disorders, University of Maryland, May, 1974.

5. Schiffer CA: Current status of granulocyte transfusion. Oncology Grand Rounds, Johns Hopkins University School of Medicine, January, 1973.

6. Schiffer CA: Efficacy and toxicities of granulocyte transfusions. Inter-Hospital Infectious Disease Conference, BCRC, April, 1974.

Serial No.: NCI-6276
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Chemotherapy of Metastatic Testicular Carcinoma

Previous Serial Number: Same

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: Michael O'Connell, M.D.

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0

Project Description:

Objectives:

To assess the efficacy of various anti-tumor chemotherapeutic agents in metastatic testicular carcinoma.

Methods Employed:

Patients are currently randomized between a combination of drugs consisting of fluorouracil, cyclophosphamide, methotrexate, and vincristine and a combination including the same drugs plus bleomycin given 12 hours after the dose of vincristine. Patients failing this protocol are randomized between adriamycin or IV methyl CCNU.

Major Findings:

Twenty-one patients have currently been entered into the study. Objective tumor regression has been observed in 16 patients. Both regimens are generally well tolerated and no major life threatening toxicity has been encountered. It is too early to determine whether the addition of bleomycin to the other agents will result in a higher remission rate or longer remission duration.

Significance to Cancer Research:

Testicular carcinomas account for a aignificant proportion of tumors in young male adults. Metastatic testicular carcinoma has been shown to be responsive

Significance to Cancer Research:

to several different classes of anti-tumor agents by this group and others. The present study shows promise of achieving results superior to those previously reported.

Proposed Course:

Continue present studies.

Honors and Awards: None

Publications:

1. Sickles, E.A., Sklansky, B.D. and Wiernik, P.H.: Benign Intrathoracic Lesions Mimicking Recurrent Cancer. JAMA 225:156-158, 1973.

Serial No.: NCI-6277
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Chemotherapy of Adult Acute Leukemia

Previous Serial Number: Same

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: William H. Greene, M.D.
Michael J. O'Connell, M.D.
Charles A. Schiffer, M.D.
Robert S. Benjamin, M.D.
Nicholas R. Bachur, M.D.
Robert Kaplan, M.D.
Sol Spiegelman, M.D.
Dwight Stickney, M.D.

Cooperating Units: Acute Leukemia Cooperative Group B
Research Microbiology, BCRC
Patient Care Pharmacy, BCRC
Section of Biochemistry, Laboratory of Pharmacology, BCRC
Section of Neurological Surgery, BCRC
Francis Delafield Hospital, New York
Burroughs-Wellcome Company, North Carolina

Man Years:

Total:	5
Professional:	2
Other:	3

Project Description:

Objectives:

1. To evaluate the effectiveness of newer chemotherapeutic agents alone and in combination against adult acute leukemia.
2. To correlate clinical results with pharmacokinetic data.
3. To define the usefulness of newer chemotherapeutic agents in Phase II trials in adult acute leukemia.
4. To identify factors of prognostic import in adult acute non-lymphocytic leukemia.
5. To study the effect of intensive anti-leukemic therapy during complete hematologic remission on the duration of remission.

Objectives: (Cont'd)

6. To identify biochemical indicators of occult disease activity in adult patients with acute non-lymphocytic and lymphocytic leukemia.

Methods Employed:

Protocol studies of various anti-tumor agents alone or in combination have been initiated. In addition muramidase has been measured by several methods, carcinoembryonic antigen by radioimmunoassay and immunoglobulin concentration in serum and other body fluids by immunoelectrophoresis. Delayed hypersensitivity has been evaluated prior to and during therapy, and after remission.

Major Findings:

I. Effect of Single Agents

A. Daunorubicin:
Daunorubicin has been found in previous studies at this institution to be the most effective single agent in the treatment of adults with acute non-lymphocytic leukemia. Consequently daunorubicin given in the dosage and schedule identified by our pharmokinetic studies with the Section of Biochemistry as the most efficacious was utilized as one arm of a controlled Phase III study in adult acute non-lymphocytic leukemia. To date 33 previously untreated patients have been entered on-study, and all are evaluable at the time of this report. The complete remission rate with this drug alone is 48% of all patients entered on protocol. If patients who died without an adequate trial are omitted from evaluation, the complete remission rate is 66%. If all patients over the age of 70 are omitted from evaluation, the complete remission rate is 52%. The median survival of patients treated with daunorubicin alone as of the date of this report is 7.0+ months. The median survival of all patients who achieved a complete response is 12.0+ months at this time. These data confirm and extend our previous observations with daunorubicin.

B. Azacytidine vs. Guanazole:
This is a Phase II study designed to determine the relative efficacy of two agents recently demonstrated to have activity in acute leukemia, namely azacytidine and guanazole. There are 7 evaluable previously treated patients on the azacytidine arm, 3 of which have achieved a complete remission. Only one partial remission was seen in 9 evaluable patients treated with guanazole. Azacytidine will be investigated further earlier in the therapy of this disease.

II. Effect of Multiple Drugs in Combination

A. Daunorubicin, Cytosine Arabinoside, Thioguanine and Pyrimethamine:
This program forms the second randomization in our current protocol for adult acute non-lymphocytic leukemia. Pyrimethamine is included in this combination of agents in an attempt to treat occult central nervous

A. Daunorubicin, Cytosine Arabinoside, Thioguanine and Pyrimethamine: (Cont'd) system leukemia at the time of diagnosis with an agent that can be given orally. Researchers at Burroughs-Wellcome and Company, Research Triangle, North Carolina have measured pyrimethamine levels in cerebral spinal fluid from our patients treated on this arm of the study and have identified the compound in adequate concentration for prolonged periods of time. Thirty-three previously untreated patients have been entered on this arm of the protocol, and all were evaluable at the time of this report. The complete remission rate for all entered patients is 45%. If patients who did not receive an adequate therapeutic trial are excluded from the evaluation, the complete remission rate is 58%. If all patients over the age of 70 are excluded from evaluation, the complete remission rate is 50%. The median survival of patients who achieved a complete response is 11.5+ months. There is evidence from this study that the administration of pyrimethamine at the time of the initial therapy in adult patients with acute non-lymphocytic leukemia is useful in treating occult meningeal leukemia.

B. Daunorubicin, Cytosine Arabinoside and Thioguanine; Cytosine Arabinoside and Thioguanine; and Daunorubicin Alone:
This study is being chaired by the principal investigator for Acute Leukemia Group B (ALGB# 7221). As of the time of this report 409 patients have been entered and 324 are evaluable. The remission rate is over 50% in each arm of the study, but it is too early to evaluate duration of remission. The study will continue to be evaluated during the current fiscal year, but it will soon be closed to entry of new patients.

C. Vincristine, Thioguanine, Pyrimethamine and Dexamethasone vs. Vincristine Thioguanine, Methotrexate and Prednisone for Remission Induction in Adults with Acute Lymphocytic Leukemia:
This program is designed to test whether or not pyrimethamine administered intially to adults with acute lymphocytic leukemia can prevent or delay the onset of meningeal leukemia in long-term survivors. Additionally, the protocol was designed to test whether or not dexamethasone, a steroid that mobilizes granulocytes might be associated with fewer infections during induction than prednisone, a steroid that inhibits granulocyte mobilization. To date, 23 patients have been entered and it is too early to evaluate the results.

III. Maintenance Therapy for Remission in Adults with Acute Non-Lymphocytic Leukemia.

A. Cytoxan and Guanazole:
All patients achieving complete remission on the daunorubicin alone arm or the daunorubicin, cytosine arabinoside, thioguanine and pyrimethamine arm of our protocol #7110 are maintained with monthly injections of cytoxan 1500 mg per sq. meter of body surface area followed in 18 hours by guanazole 25 grams per sq. meter in a 24 hour IV infusion. It is hoped that cells in G_0 phase might be recruited into the cell cycle

A. Cytoxan and Guanazole: (Cont'd)

by the agent cytoxan. Subsequent treatment with guanazole, an S-phase active agent, might then accomplish a greater degree of cell kill than if used alone. The combination of these agents used in this way might thereby prolong the duration of remission over that which is customarily achieved in this disease. At present the median duration of first remission in both arms of the study is approximately 7 months with many patients still in remission below the median. This figure compares favorably with the 2.3 months median duration of unmaintained remission seen in a previous study here utilizing daunorubicin or POMP.

IV. Biochemical Indicators of Disease Activity in Acute Non-Lymphocytic Leukemia.

A. Serum and Urine Muramidase Studies:

Our studies in serum and urine muramidase activity in adults with acute non-lymphocytic leukemia continue. We continue to confirm the utility of serum muramidase levels in differentiating between acute monocytic, myelomonocytic, and myelocytic leukemia. In addition we continue to observe that serum muramidase levels accurately reflect disease activity in a large proportion of patients with acute non-lymphocytic leukemia. It has also become evident that patients with initially elevated serum muramidase levels who achieve complete remission and normal serum muramidase levels have a longer remission than those who achieve hematologic and clinical remission but maintain elevated serum muramidase levels. Urinary muramidase has been demonstrated to be nephrotoxic and to be responsible for the hypokalemia seen in many patients with acute leukemia.

B. Carcinoembryonic Antigen:

We have observed that approximately 30% of adult acute non-lymphocytic leukemia or acute lymphocytic leukemia have abnormal levels of carcinoembryonic antigen in their serum. Unfortunately, these levels do not correlate well with disease activity. Most patients undergoing therapy have a rise for weeks or months in their plasma CEA whether or not they respond to therapy. In addition, when patients relapse, the presence or absence of carcinoembryonic antigen in their plasma at that time does not correlate with findings at the time of presentation.

C. Serum Immunoglobulin Concentrations in Patients with Acute Non-Lymphocytic Leukemia:

We have measured quantitatively serum IgA, IgD, IgE, and IgM in patients with acute non-lymphocytic leukemia prior to therapy, during therapy, during remission and relapse. Most patients with acute non-lymphocytic leukemia present with decreased levels of IgG in their serum. When complete remission is achieved these levels become normal. Investigation continues into the question whether or not serial immunoglobulin levels might be useful in determining the disease status in a patient with acute non-lymphocytic leukemia.

D. Reverse Transcriptase in Leukemic Cells:
This enzyme of presumed viral origin is being looked for in cells and plasma from our leukemia patients by Dr. Sol Spiegelman, New York. Results are preliminary at this point. 22 patients have been studied prior to therapy and the enzyme was present in the white cells of each. In addition, most patients in complete remission continue to have the enzyme detectable in their white cells.

E. Cell-Mediated Immunity in Acute Non-Lymphocytic Leukemia:
Cell-mediated immunity in acute non-lymphocytic leukemia, either at initial presentation or in subsequent relapse, was determined among 64 patients by recall-antigen skin testing and in 37 patients by attempted sensitization with dinitrochlorobenzene (DNCB). Thirty (46.9%) of the 64 skin-test batteries displayed reactivity to at least one established antigen, and 17 (51.5%) of the 33 evaluable patients were successfully sensitized with DNCB. Twenty-six (78.8%) of the 33 patients tested with both DNCB and recall antigens reacted to either one or both. The leukemic patient's subsequent prognosis, in terms of attainment of complete remission of his or her leukemia, duration of remission, and survival could not be correlated with the pretherapy demonstration of intact recall-antigen or DNCB cell-mediated immunity.

Summary of Major Findings:

1. Daunorubicin alone may be as effective an inducer agent for adult acute non-lymphocytic leukemia as the other combinations of agents tested. Since remissions are generally achieved more quickly with daunorubicin alone than with combinations of agents, daunorubicin may ultimately prove to be superior for remission inducation when compared to other presently used combinations of agents.
2. Pyrimethamine may be useful in meningeal leukemia prophylaxis in adults with acute non-lymphocytic leukemia.
3. Serum muramidase levels are useful in following disease activity in adult acute non-lymphocytic leukemia.
4. Carcinoembryonic antigen is present in increased amounts in the minority of patients with acute non-lymphocytic leukemia. However, serial determinations are useless in following disease activity.
5. Cell-mediated immunity in leukemia patients prior to therapy may not be important in determining response.
6. The presence of reverse transcriptase in the white cells of leukemia patients in remission may indicate the necessity for further induction therapy.

Significance to Cancer Research:

These studies indicate continued improvement in our ability to arrest disease activity in adults with acute non-lymphocytic leukemia in an ever increasing number of patients. In addition, biochemical parameters are being identified that reflect accurately disease activity in many patients with acute non-lymphocytic leukemia. The identification of such factors may lead to more rational therapy in the future. Demonstration of reverse transcriptase in

Significance to Cancer Research: (Cont'd)

leukemic cells may have etiologic significance.

Proposed Course of Project:

These investigations will be continued through the fiscal year.

Honors and Awards: None.

Invited Lectures: Peter H. Wiernik, M.D.

1. University of Maryland School of Medicine, November, 1973.
2. St. Joseph's Hospital, Baltimore, Maryland, December, 1973.
3. Greater Baltimore Medical Center, February, 1974.
4. Johns Hopkins University School of Medicine, January, 1974.
5. Bon Secours Hospital, Baltimore, Maryland, April, 1974.
6. Visiting Professor; West Virginia University School of Medicine, Morgantown, June, 1974.
7. Visiting Professor; University of Iowa, Iowa City, December, 1973.
8. Grand Rounds, University of Florida School of Medicine, Gainesville, April, 1974.
9. Grand Rounds, Emory University, Atlanta, July, 1973.

Publications:

1. Rosenoff, S.H., Canellos, G.P., O'Connell, M. and Wiernik, P.H.: Mediastinal Adenopathy in Granulocytic Leukemia. Arch. Int. Med., 1974 (in press).
2. Greene, W.H., Schimpff, S.C., Wiernik, P.H.: Cell-mediated immunity in acute nonlymphocytic leukemia: relationship to host factors, therapy, and prognosis. Blood 43: 1, 1974.
3. Wallace, H.J., Holland, J.F., Glidewell, O.J., Ellison, R.R., Weil, M., Carey, R.W., Schwartz, J.M., Hoagland, H.C., Henderson, E.S., Wiernik, P., Yates, J.W.: Therapy of acute myelocytic leukemia - Acute Leukemia Group B Studies. Minerva Med., in press, 1974.
4. Sklansky, B.D., Jafek, B.W., Wiernik, P.H.: Otolaryngologic manifestations of acute leukemia. Laryngoscope 84: 210, 1974.
5. Wainer, R.A., Wiernik, P.H., Thompson, W.L.: Metabolic and therapeutic studies of an acute leukemia patient with severe lactic acidosis. Amer. J. Med. 55: 255-260, 1973.

Serial No.: NCI-6278
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Determination of Serum Alpha Fetoprotein and Plasma Carcinoembryonic Antigen, Regan Isoenzyme, and Other Markers in Patients with Malignant Disease.

Previous Serial Number: Same

Principal Investigators: Peter H. Wiernik, M.D. and Robert Belliveau, M.D.

Other Investigators: Robert J. Esterhay, Jr., M.D.
K.R. McIntire, M.D.

Cooperating Units: Laboratory of Cell Biology, NCI
Hoffman-La Roche, Medical Research Department, Nutley, New Jersey
Section of Neurological Surgery, BCRC
Pathology Department, USPHS Hospital, Baltimore

Man Years:

Total:	1 1/2
Professional:	1
Other:	1/2

Project Description:

Objectives:

To determine whether or not carcinoembryonic antigen and alpha fetoprotein are present in the serum or plasma of patients with a variety of malignant diseases. In addition, to determine whether or not serial determinations of alpha fetoprotein and carcinoembryonic antigen in patients with initially elevated levels are useful in following disease activity.

Methods Employed:

Carcinoembryonic antigen, alpha fetoprotein, and Regan isoenzyme are determined by radioimmunoassay. Patients are investigated prior to any therapy and those with positive results are followed serially throughout their course.

Major Findings:

1. Carcinoembryonic antigen is present in the plasma of patients with a variety of neoplasms including hematologic malignancies, brain tumors, neuroblastoma, gastrointestinal tumors and others.
2. In some patients plasma carcinoembryonic antigen levels accurately reflect disease activity. In others, the levels correlate better with complications, such as infection.
3. Alpha fetoprotein elevations in sera are not specific for patients with hepatocellular carcinoma. Elevated alpha fetoprotein is found in about 25% of patients with embryonal cell carcinoma of the testes and teratocarcinoma of the ovary. In addition, a rare patient with gastric carcinoma with elevated serum alpha fetoprotein can be identified.
4. Regan isoenzyme is present in the serum of a significant number of patients with malignant lymphoma.

Significance to Cancer Research:

The identification of biochemical parameters that may signal the presence of occult tumors is of prime importance to optimal therapy of cancer.

Proposed Course:

Continue present studies.

Honors and Awards: None

Publications:

1. Belliveau, R.E., Wiernik, P.H., Abt, A.B.: Liver enzymes and pathology in Hodgkin's disease. Cancer, in press, 1974.
2. Belliveau, R.E., Wiernik, P.H., Sickles, E.A.: Blood carcinoembryonic antigen, Regan isoenzyme, and human chorionic gonadoropin in a man with primary mediastinal choriocarcinoma. Lancet 1: 22, 1973.
3. Belliveau, R.E., Yamamoto, L.A., Wassell, A.R., Wiernik, P.H.: Regan isoenzyme in patients with hematopoietic tumors. Amer. J. Clin. Path., in press, 1974.

Serial No.: NCI-6279
1. BCRC
2. Sections of Medical Oncology
   and Biochemistry
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Initial Clinical and Pharmacological Evaluation of Adriamycin

Previous Serial Number: NCI-6279

Principal Investigator: Robert Benjamin, M.D.

Other Investigators: Peter H. Wiernik, M.D.
Nicholas R. Bachur, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives:

1. To determine the effectiveness and toxicity of adriamycin given by a single intermittent high-dosage schedule in a variety of malignancies.
2. To study the pharmacokinetics of adriamycin in these patients.
3. To make clinical and pharmacologic correlations.
4. To define the conditions under which dosage reduction will result in therapeutic plasma levels.

Methods:

Any patient with an unresectable neoplasm not suitable for other BCRC protocols is eligible for the study. Standard dosage is 60 mg/M^2 repeated in 3-4 weeks after resolution of toxicity from the previous dose. In patients with severe liver disease BSP>15% and/or bilirubin>3 mg%) dosage is reduced by 75%. In patients with moderate liver disease (BSP 9-15%, bilirubin 1.2-3 mg%) dosage is reduced by 50%. Serial samples of plasma and urine will be collected on selected patients and analyzed by spectrophotofluorometry and thin-layer chromatography for drug and metabolites.

Major Findings:

Clinical Efficacy

Eighty--two of 96 patients are evaluable. Responses have been seen in a variety of tumors. In 26 patients with hematologic neoplasms, 4 obtained complete remission (2 nodular PDL lymphomas, 1 diffuse histiocytic lymphoma, 1 ALL); 11 obtained partial remission, and 6 improved. The remission rate in pure and mixed histiocytic lymphomas (CR + PR = 5/7 = 86%) and lymphocytic lymphomas (CR + PR = 5/8 = 62%) is particularly noteworthy. In sarcomas, complete remissions have been seen in patients with myxofibrosarcoma and soft-tissue Ewing's sarcoma. Partial remissions were most common in rhabdomyosarcoma (4/5) but were also seen in osteosarcoma, leiomyosarcoma, mesothelioma, and giant-cell tumor of bone. Improvement was noted as well in patients with liposarcoma and malignant fibrous histiocytoma. Overall remission rate (CR + PR) was 10/25 or 40%, response rate was 16/25 or 64%. In carcinomas, best results were seen in tumors of lung, breast, and testis. No complete remissions were seen, but 5/10 with lung cancer (2-adenocarcinoma, 1 large-cell undifferentiated carcinoma, 2 small-cell carcinoma) had partial remissions and 2 (1-adenocarcinoma, 1-squamous carcinoma) improved. In breast cancer, 3/5 had partial remissions and the other two improved. In testicular carcinoma, 1/5 had partial remission and 2 others improved. Partial remission was also seen in 1 patient with anaplastic carcinoma of the thyroid, and improvement was seen in patients with neuroblastoma, malignant teratoma of the ovary, and adenocarcinoma of unknown primary site. Overall remission rate for patients with carcinomas was 10/31 = 32%; response rate was 19/31 = 61%. Median duration of remission was 11+ weeks for hematologic malignancies, 18+ for sarcomas and 15+ for carcinomas. Survival in sarcomas was significantly prolonged. Six patients with solid tumors showed CNS progression despite systemic response.

Clinical Toxicity

Major toxicity included bone marrow suppression, mucositis, and cardiomyopathy. Cardiac toxicity was questionably present in only 1 case (1%). Mucositis was infrequent (9%) due to the intermittent single high-dosage schedule. Hematologic toxicity characterized by leukopenia was present in 86%. Thrombocytopenia (in 3/49) and severe leukopenia (in 1/49) was rare in the absence of 3 risk factors--prior radiotherapy (but not chemotherapy), bone marrow infiltration, and severe liver disease without dosage reduction. In 8 patients with liver disease, but without dosage reduction, toxicity was severe--all had marked pancytopenia, 3/8 (38%) died because of marrow depression, and 3/8 (38%) had severe mucositis.

Pharmacokinetics

Mean long plasma half-life of total drug fluorescence was 26.7 hours; relative volume of distribution was 334 L/M^2. For adriamycin itself the mean long half-life was 16.7 hours; relative volume of distribution was 1780 L/M^2. Cumulative urinary excretion of drug and metabolites was 5.7% of the administered dose

Pharmacokinetics (Cont'd)

of which about 60% was adriamycin. In plasma, levels of adriamycin were low compared with the level of pooled metabolites. In patients with severe liver disease treated with full dosage, plasma levels of drug and metabolites were 4-5 times normal, and elimination was irregular and was greatly delayed. In patients with liver disease treated with reduced dosage, levels of drug and metabolites approached those in patients without liver disease treated with full dosage. Neither adriamycin nor its metabolites were detectable in CSF.

Clinical-Pharmacologic Correlations

Adriamycin dosage need not be adjusted in the presence of renal insufficiency but 50-75% reductions in dosage are necessary to prevent life-threatening toxicity in patients with severe liver disease. When so treated, these patients show response rates, toxicity, and plasma levels similar to those in patients without liver disease treated with full dosage. Due to limited penetration into the CNS, adriamycin may not be effective in controlling CNS metastases from sensitive tumors. The prolonged plasma levels after a single dose make repeated doses unnecessary and more toxic, especially with regard to mucositis.

Significance to Cancer Research:

The basic pharmacokinetics of adriamycin in man has been elucidated. Utilizing the pharmacologic data as the basis of a clinical dosage schedule, we have devised the intermittent single high-dosage regimen described. We have singled out 3 risk factors for the development of severe toxicity and devised a way of eliminating the excess toxicity in patients with liver disease without sacrificing drug efficacy. When adriamycin is given according to our regimen, the therapeutic index is significantly increased, for our results are at least as good as any others reported and our toxicity significantly lower.

Proposed Course:

Study completed.

Honors and Awards:

J.D. Lane Award, United States Public Health Service, 1973.

Publications:

1. Robert S. Benjamin, Charles E. Riggs, Jr., and Nicholas R. Bachur: The pharmacokinetics and metabolism of adriamycin in man. Clin. Pharmacol. Therap. 14: 592-600, 1973.
2. Benjamin, R.S., Wiernik, P.H., and Bachur, N.R.: Adriamycin chemotherapy - efficacy, safety, and pharmacologic basis of an intermittent single high-dosage schedule. Cancer 33: 19, 1974.

Serial No.: NCI-6280
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Combination Chemotherapy for Stage III-B and IV Hodgkin's Disease

Previous Serial Number: Same

Principal Investigators: Peter H. Wiernik, M.D.
Michael J. O'Connell, M.D.

Other Investigators: None

Man Years:

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives:

To determine whether a combination of cyclophosphamide, vinblastine, procarbazine, and prednisone might prove efficacious in the therapy of advanced Hodgkin's disease.

Major Findings:

Thirty-two patients have currently been entered on this study. Twenty-four have completed therapy and are evaluable. Among the 11 previously untreated patients, 8 (79%0 have achieved complete remission. Seven of 13 previously treated patients (54%) have achieved complete remission. Extensive prior therapy with both radiation therapy and chemotherapy decreased the response rate (none of the 3 patients in this category had a useful response). It is too early to define the duration of remission at this time.

Severe leukopenia was seen in two of 11 previously untreated patients and 6 of 13 previously treated patients. Thrombocytopenia was less severe with only 2 patients (all previously treated) experiencing severe thrombocytopenia. Three episodes of hemorrhagic cystitis have been observed. Only 1 patient has experienced neurologic complications. Overall, the toxicity of this regimen appears comparable to other chemotherapy combinations currently used to treat Hodgkin's disease.

Significance to Cancer Research:

Although many combinations of effective agents have been identified for the treatment of advanced Hodgkin's disease, in the best hands 20% of patients fail to respond significantly. If this study will result in a regimen that produces a longer duration of response than obtained with other regimens, the therapy of disseminated Hodgkin's disease will have been improved.

Proposed Course:

Continue present study.

Honors and Awards: None

Publications: None

Presentations: None

Serial No.: NCI-6293
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: A Teaching and Consultation Service in Medical Oncology

Previous Serial Number: Same

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: William Greene, M.D.
Robert Esterhay, Jr., M.D.
Stephen Schimpff, M.D.
Michael J. O'Connell, M.D.
Charles Schiffer, M.D.
John Levi, M.D.

Cooperating Units: University of Maryland School of Medicine
Johns Hopkins University School of Medicine
Greater Baltimore Medical Center
Maryland General Hospital
The Bryman Medix School

Man Years:

Total:	1 1/2
Professional:	1 1/2
Other:	0

Project Description:

Objectives:

To teach the theory and practice of medical oncology to medical students and house officers. To acquaint such individuals with newer aspects of chemotherapy, radiotherapy and surgery as they apply to neoplastic diseases. To provide a consultative service for difficult patients with neoplastic diseases for other physicians in the Baltimore area free of charge. To teach basics of cancer nursing to physician assistant students.

Methods Employed:

Second year medical students from the University of Maryland are assigned to this service on a regular basis for their physical diagnosis class. Students from the University of Maryland and Johns Hopkins are assigned here during elective periods for a clinical clerkship in medical oncology. In addition house officers from the Greater Baltimore Medical Center and Maryland General Hospital have been assigned here for elective periods of their house officer year. University of Maryland Assistant Residents in Medicine rotate through the Section of Medical Oncology on a regular basis. Physicians in the community refer patients here for an out-patient consultation. If the patient meets the qualifications for one of our protocol studies, he is accepted in transfer and admitted to the BCRC. If not, the patient is completely evaluated as an out-patient and returned to his physician with specific recommendations for therapy. Physician assistant students from the Bryman School are assigned to the wards and out-patient clinics of the BCRC for instruction in the basics of nursing in oncology.

Major Findings:

1. Students, house officers, and physician assistants who have rotated through the BCRC are recognizing that medical oncology is a bona fide subspecialty of internal medicine. They have learned that patients with neoplastic diseases require special care in order to achieve good results.
2. It has been suggested by some local private physicians that the general care of patients with neoplastic diseases in the community has advanced because of our consultation service.

Significance to Cancer Research:

The dissemination of information concerning the latest techniques in diagnosis and therapy of cancer patients is of paramount importance in advancing the attack on this disease. By training potential physicians at an early time, such as during their student years and house officer years, such information will be brought to the community in a more orderly and useful way than is sometimes possible. By seeing patients in consultation we have the opportunity to educate the patient's physician as to modern techniques of management for a given neoplastic disease. Both of these teaching functions we engage in are of significant import in elevating the level of care available to patients with cancer in this area.

Proposed Course:

Continue project.

Honors and Awards: None

Publications: None

Lectures:

1. Peter H. Wiernik, M.D.: Attending Physician, General Medical Ward, Greater Baltimore Medical Center, February, 1973
2. Peter H. Wiernik, M.D. and Stephen C. Schimpff, M.D.: Teaching sessions on acute leukemia. American Society of Hematology Meeting, December, 1973

3. Peter H. Wiernik, M.D. and Michael D. Walker, M.D.: "Answers to Your Questions About Cancer", WFBR radio, November, 1973.
4. Peter H. Wiernik, M.D. and Stephen C. Schimpff, M.D.: "Advances in Cancer Therapy", WMAR TV, March, 1974.
5. Peter H. Wiernik, M.D.: "What's New in Cancer Research", WBAL TV, March, 1974.
6. Michael J. O'Connell, M.D. and Stephen C. Schimpff, M.D.: "Cancer Chemotherapy", Annual Meeting of Read's Pharmacists, April, 1974.

Serial No.: NCI-6295
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Census: A Remote Access Patient Information Storage and Retrieval System

Previous Serial Number: NCI-6039

Principal Investigators: William L. Robinson
Robert J. Esterhay, Jr., M.D.

Other Investigators: Paul Chang, M.D.

Cooperating Units:
Section of Neurological Surgery, NCI, BCRC
Nursing Service, NCI, BCRC
Section of Microbiology, NCI, BCRC
Patient Care Pharmacy Service, NCI, BCRC
Administrative Office, NCI, BCRC
Section of Medical Oncology, NCI, BCRC

Man Years:
Total: 3
Professional: 3
Other: 0

Project Description:

Objectives:

1. To compile, update, and retrieve from a data base of medically relevant information on cancer patients that is centralized, readily accessible, and maintained by a single non-technical individual.

2. To generate a daily report on all in-patients and their locations, along with their vital statistics and current therapy data, to be distributed to all involved in the tasks of clinical research, patient care and administration.

3. To develop flexible data retrieval programs which generate a variety of useful patient summaries keyed on any of several patient parameters.

Methods Employed:

1. Information is compiled and recorded daily in a data input log by a medical secretary.
2. A computer program accepts the input datum from the secretary, checks for logical content and accuracy, and records it on the data base.
3. A second computer program generates a Daily Census Report which reflects the updated status of the data base.
4. The report is then distributed to the scientific and professional staff, remote contracting laboratories, and administrative office.
5. The programs and data base are executed and stored on a commercial time-sharing service.
6. Mass memory disk files and remote terminals form the storage and access mediums.

Major Findings:

The Daily Census Report has been utilized by all sections and services of the Cancer Center.

1. Clinical Associates: Used at evening sign-out rounds as a checklist of patients with special problems that may require extra care overnight.
2. Admitting Officer: Used to assign new patients to their physicians and to distribute the patient load evenly among the physicians.
3. Nursing Service: Used to assign patients to vacant beds.
4. Section of Microbiology: Provides the laboratory staff and outside contracting laboratories with relevant information on patients whose cultures are being analyzed. The permanent records of all beds a patient has occupied provide a useful epidemological tool in detecting the spread of infectious organisms. The data base is also accessed by computerized microbiology reporting systems at the BCRC. (See Project Report NCI-6039).
5. Patient Care Pharmacy Service: Used to fill the unit drug cart patient trays.
6. The master retrieval programs are most useful to the physician performing a chart review to evaluate treatment protocols and who requires listings

Major Findings: (Con'd)

of the patient population which are sorted by status, cancer diagnosis, research protocol, etc.

7. Several programs have been written to produce administrative and accounting tabulations of the data stored on the system.

Significance to Cancer Research:

The content and flexibility of the retrieval summaries generated by the Census System have provided the Research Center with a valuable "index" to patient records. The daily routine of update and audit have eliminated the inaccuracies and incompleteness of previous disjoint and ill-kept data bases.

Proposed Course:

Three years of continuous operation of the Census System have provided BCRC with a substantial data base of information on cancer patients. Future developments will concentrate on effective utilization of the information thus obtained. These developments will be in the form of: 1) special purpose retrieval programs to serve specific research applications, 2) programs to correlate census data with data compiled by other data systems at the BCRC.

Honors and Awards: None

Lectures: None

Presentations:

1. William L. Robinson and Robert J. Esterhay, Jr., M.D., Tenth Symposium on Biomathematics and Computer Science in the Life Sciences, Houston, Texas, March 29, 1973, "Census: A Remote-Access Patient Information Storage and Retrieval System".

Publications: None

Serial No.: NCI-6297
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Streptozotocin Therapy for Malignant Carcinoid and Pancreatic Islet Cell Tumors.

Previous Serial Number: Same

Principal Investigators: Michael J. O'Connell, M.D.
Peter H. Wiernik, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/8
Professional:	1/8
Other:	0

Project Description:

Objectives:

To evaluate the activity of streptozotocin in a Phase II study against malignant carcinoid tumors and pancreatic islet cell tumors.

Methods Employed:

Patients with histologically documented malignant carcinoid tumor or pancreatic islet cell tumor are treated with streptozotocin in a dose of 1.5 grams per meter squared on a weekly basis, providing that baseline renal function is adequate.

Major Findings:

Only one patient has been entered into the study, and he is still undergoing therapy.

Significance to Cancer Research:

Initial reports appearing in the literature suggested a very high order of activity for streptozotocin against both pancreatic islet cell tumors and malignant carcinoid tumors. Our initial experience suggested a less optimistic outlook with regard to metastatic carcinoid tumors. Therefore, this project was undertaken to further define the role of streptozotocin in the

Significance to Cancer Research: (Cont'd)

therapy of thses disorders.

Proposed Course:

Continue present studies.

Honors and Awards: None

Publications: None

Serial No.: NCI-6298
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Streptozotocin in the Therapy of Lymphoproliferative Disorders

Previous Serial Number: Same

Principal Investigators: Michael J. O'Connell, M.D.
Peter H. Wiernik, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

1. To evaluate the activity of streptozotocin against various lympholiferative malignancies.
2. To compare the relative efficacy of a weekly vs. a cyclical daily dosage schedule.

Methods Employed:

Patients with histologically documented Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphatic leukemia, or chronic lymphatic leukemia are eligible for study regardless of previous therapy provided the pre-treatment renal function is adequate. Patients are randomly assigned to either regimen A or regimen B. Regimen A consists of 5 consecutive daily doses of streptozotocin in a dose of 500 mg/M^2 to be repeated every 2 weeks. Regimen B prescribes weekly doses of streptozotocin at a dosage level of 1.5 $grams/M^2$.

Major Findings:

Ten patients have been entered into the study, and 9 are currently evaluable. All patients had received extensive previous therapy and were refractory to conventional chemotherapeutic agents. Two of 5 patients receiving streptozotocin daily for 5 days showed evidence of objective tumor regression, whereas 0 of 4 patients receiving streptozotocin weekly showed evidence of tumor response. Both patients who showed objective improvement had lympho-

Major Findings: (Cont'd)

cytic lymphomas. The number of patients studies is too samll to compare the relative efficacy of the 2 dosage schedules.

Significance to Cancer Research:

Preliminary clinical trials have suggested that streptozotocin has activity against a variety of lymphoproliferative disorders. Since this agent is not significantly myelosuppressive, it may find a role in the management of these diseases when patients either become refractory to conventional modes of therapy or are unable to tolerate the myelosuppression resulting from such therapy. Therefore, this study was designed to more clearly define the spectrum of activity in patients with a variety of malignant lymphoproliferative conditions.

Proposed Course:

Continue present studies.

Honors and Awards: None

Publications:

1. Schein, P., O'Connell, M., Blom J., et al: Clinical Antitumor Activity and Toxicity of Streptozotocin. Cancer (In press).

Presentations:

1. Schein, P.: Clinical Antitumor Activity and Toxicity of Streptozotocin. National Meeting of A.A.C.R. Houston, Texas, March 29, 1974.

Serial No.: NCI-6299
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Therapy of Patients with Metastatic Sarcoma

Previous Serial Number: None

Principal Investigator: Paul Chang, M.D.
Peter H. Wiernik, M.D.

Other Investigators: Stephen Schimpff, M.D., Mark Green, M.D., Nicholas R. Bachur, M.D., Ph.D.

Cooperating Units: Biochemistry Section, BCRC

Man Years:

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives:

A) Adriamycin vs. Adriamycin + Streptozotocin for Previously Untreated Patients with Metastatic Sarcoma - Although uncommon in adults, soft tissue and bone sarcomas constitute a major group of fatal malignancies in children. Until recently, Ewing's sarcoma and childhood embryonal rhabdomyosarcoma were the only tumors of this group which were more than occasionally responsive to chemotherapy. Recent studies at BCRC and elsewhere have demonstrated significant, clinically useful responses in 25-40% of patients treated with adriamycin with mild marrow and minimal mucosal toxicity. Phase II studies at this center have also found anti-sarcoma activity with streptozotocin, a non-myelosuppressive agent. Therefore, a randomized clinical trial has been undertaken comparing the efficacy of adriamycin alone to adriamycin + streptozotocin given simultaneously in previously untreated patients with inoperable or metastatic sarcomas without evidence of central nervous system spread. Patients who have or develop CNS metastases will be treated according to part B of this project.

The primary objective of this study is to develop more adequate therapy of metastatic sarcoma without intolerable toxicity. A secondary objective is

Objectives (con'd):

to further evaluate the efficacy of streptozotocin in this group of tumors.

B) Intravenous Methyl-CCNU for Advanced Sarcoma - The Phase I study of methyl-CCNU administered intravenously (IV methyl-CCNU) at the BCRC last year demonstrated stabilization or regression of tumor in three of nine previously extensively treated patients with metastatic sarcoma. All patients were previously shown to be refractory to adriamycin. Pharmacokinetic studies of this highly lipid-soluble agent demonstrated a significant penetration of drug radiolabelled C^{14} into the CSF. Thus, a Phase II study with IV methyl-CCNU has been started in patients with metastatic sarcomas refractory to adriamycin and in patients with central nervous system metastases from sarcomas.

C) Use of Etiocholanolone to Determine Bone Marrow Reserves - Etiocholanolone-induced mobilization of granulocytes may be useful in assessing marrow tolerance to drug therapy. Serial measurement of etiocholanolone stimulated granulocyte mobilization in patients receiving recurrent doses of single agent might also be an aid in assessment of subclinical cumulative bone marrow toxicity.

Major Findings:

A) Adriamycin vs. Adriamycin + Streptozotocin - Two of eight evaluable patients treated with adriamycin only have had partial response of their disease (over 50% decrease in tumor surface area) with at least stabilization of previously advancing disease in two others. One of six patients receiving the combination of adriamycin and streptozotocin has had a partial response, with at least stabilization in an additional two patients. Toxicity has been acceptable in both groups, but may be greater in the group receiving the combination.

The numbers are too small to show a difference between the two arms. Pharmacokinetic studies are now also being done to determine if serum levels of either drug are affected by simultaneous administration of the other.

B) Intravenous Methyl-CCNU for Advanced Sarcoma - Twelve patients, eleven of whom are evaluable, have received the drug on this study. One has had a partial response which continues after five months while two others have had lesser responses with at least stabilization of disease. Aside from significant myelosuppression, toxicity has been minimal. More patients are needed before these results can be meaningful.

C) Assessment of Bone Marrow Reserves in Patients with Sarcomas - Seven patients treated with IV methyl-CCNU have had pre-treatment etiocholanolone tests done. Only one of these was abnormal, so that insufficient numbers have as yet accumulated for comparison.

Serial No.: NCI-6299

Proposed Course: Continue all studies.

Significance to Cancer Research: These studies represent a concerted effort to advance the therapy of metastatic sarcoma by taking advantage of non-overlapping toxicity in clinically active agents to initiate combination chemotherapy. Moreover, it is hoped that more accurate prognosis and assessment of toxicity will be gained via the ancillary studies.

Honors and Awards: None

Presentations:

I. Dr. Greene

Proposed Additional Studies in Sarcoma, 4th Conjoint Meeting of the Status of Clinical Studies at the National Cancer Institute, Annapolis, Maryland, January, 1973.

Serial No.: NCI-6900
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Radiation Alone vs. Radiation Therapy Followed by Combination Chemotherapy in the Treatment of Hodgkin's Disease

Principal Investigators: Michael J. O'Connell, M.D., Peter H. Wiernik, M.D.

Other Investigators: Kirkland C. Brace, M.D., Roger Byhardt, M.D.

Cooperating Units: Radiotherapy Service, BCRC

Man Years: (July 1, 1973 - June 30, 1974)

Total:	1 1/2
Professional:	3/4
Other:	3/4

Project Description:

Objectives:

To determine whether the addition of combination chemotherapy (MOPP) administered following radiation therapy to all involved lymph node regions as determined by staging laparotomy is superior to radiation therapy alone to involved plus adjacent lymph node regions in patients with Hodgkin's disease. This study was designed to compare in a randomized fashion the two best arms of our previous project (NCI-6230).

Methods Employed:

Previously untreated patients with less than stage IIIB Hodgkin's disease on the basis of exploratory laparotomy were randomized to receive either radiation therapy alone to involved plus adjacent lymph node regions, or radiation therapy to involved lymph node regions only followed by combination chemotherapy (MOPP) by card selection.

Major Findings:

Eighty-seven patients have currently been randomized on protocol, 45 to receive extended field radiation alone, and 42 to receive combined modality treatment. There have been 6 protocol violations, 5 patients are still undergoing initial therapy, and 4 patients (2 in each group) have failed to achieve remission from initial radiation therapy. Three of the 4 patients have died and the fourth has disseminated Hodgkin's disease and is receiving chemotherapy. The remaining 72 patients (41 treated with radiation alone and

Major Findings: (Cont'd)

31 receiving radiation and chemotherapy) achieved remission and are currently evaluable. There was no significant difference in age, sex, histologic subtype, incidence of mediastinal Hodgkin's disease, "B" symptoms, extranodal extension or pathological stage of disease between study groups. There was a somewhat higher incidence of splenic involvement in patients with stage III Hodgkin's disease treated with radiation therapy and adjuvant chemotherapy.

Nine of 41 patients treated with radiation alone have suffered recurrent Hodgkin's disease, compared to only 1 of 31 treated with radiation therapy and chemotherapy ($P<.05$). There has been a stastistically significant improvement in complete remission duration (disease free survival) in patients receiving combined modality therapy ($P<.005$). The only three deaths observed in patients completing initial therapy occurred in stage III Hodgkin's disease treated with radiation alone. As yet, there is no statistically significant difference in survival.

Proposed Course:

Continue present study.

Honors and Awards: None

Publications: None

Presentations:

1. O'Connell, M.J.: Adjuvant Chemotherapy in the Management of Hodgkin's Disease. University of Maryland School of Medicine, Department of Internal Medicine, March 4, 1974.
2. O'Connell, M.J.: Radiotherapy Followed by Chemotherapy in Hodgkin's Disease Confined to Lymph Nodes: Preliminary Results of a Prospectively Randomized Clinical Trial. National Meeting of the American Society of Clinical Oncology, Houston, Texas, March 27, 1974.

Serial No. NCI-6913
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Diagnosis of Unexplained Pulmonary Infiltrates in Cancer Patients. A Pilot Study Utilizing Transtracheal Bronchial Brushing.

Previous Serial Numbers: None

Principal Investigator: Joseph Aisner, M.D.

Other Investigators: Stephen C. Schimpff, M.D., Viola M. Young, Ph.D.
Peter H. Wiernik, M.D.

Cooperating Units: Research Microbiology, BCRC, and Pathology and Radiology Departments, USPHS Hospital.

Man Years (July 1, 1973 - June 30, 1974)

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives

The cancer patient is frequently immunologically compromised and myelosuppressed. Such patients often develop pulmonary complications, especially infection. Such pulmonary infections are a contributing factor in the demise of approximately half of these patients. A major reason for the high mortality lies in difficulty in establishing an etiologic agent and thereby providing appropriate antimicrobial therapy. Several investigators have emphasized the low yield of noninvasive culture techniques in pneumonia occurring in patients with hematological malignancies. Furthermore, myelosuppressed patients frequently carry gram negative organisms and fungi in their nasooropharynx which will contaminate expectorated sputum. The purpose of this study is to establish a method for diagnosing unexplained pulmonary infiltrates where conventional methods have been unsuccessful. This study utilizes a transtracheal approach with selective bronchial brushing, and percutaneous lung biopsy when other methods have failed.

Major Findings

As of February 1974, 22 patients with diffuse or nodular pulmonary infiltrates have undergone transtracheal bronchial brushing. Conventional methods, including transtracheal aspiration, failed to establish a diagnosis in all patients. Many of the patients were on broad spectrum antibiotics for fever, complicating subsequent interpretation. Five of six patients subsequently proven to have infection were diagnosed by brushing. One additional patient was shown to have metastatic sarcoma. Diagnosis established by the brushing procedure included: *Aspergillus* pneumonia, *T. glabrata* pneumonia, anaerobic bacterial pneumonia, and metastatic sarcoma.

There was no mortality associated with the procedure. Morbidity was acceptable including three cases of pneumothorax and one case of mediastinal emphysema. Despite pancytopenia, bleeding was not a problem.

Proposed Course

Continue investigation.

Honors and Awards

None

Publications

1. Sickles, EA, Young, VM, Greene, WH, Wiernik, PH: Pneumonia in acute leukemia. Ann. Int. Med. 79:528-534, 1973.

Presentations

1. Aisner, J: Determination of the etiologic agent of pneumonitis with transtracheal selective bronchial brushing, Inter-Hospital Infectious Disease Conference, Baltimore, Md. April 25, 1974.

Serial No. NCI-6914
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Intensive supportive care alone versus intensive supportive care plus radiotherapy for localized inflammatory lesions in patients with acute leukemia.

Previous Serial Numbers: None

Principal Investigator: John A. Levi, M.D.

Other Investigators: Stephen C. Schimpff, M.D., Peter H. Wiernik, M.D.

Cooperating Units: Section of Radiotherapy.

Cooperating Investigators: Roger Byhardt, M.D., Kirkland Brace, M.D.

Man Years:

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives:

To define the optimal management of these localized lesions in patients with acute leukemia and in particular to determine in a controlled fashion whether low-dose irradiation therapy administered to sites of local inflammation and infection will facilitate the healing process and prevent the development of disseminated infection with its associated morbidity and mortality.

Localized irradiation therapy has been utilized in the past in uncontrolled studies in patients with leukemia and results achieved have suggested some benefits to be derived from its use.

Methods Employed:

Patients with acute leukemia who develop a localized superficial inflammatory lesion which is not secondary to a previously proven disseminated infection are randomized to receive local care alone to these lesions or local care plus 400 rads of megavoltage irradiation directed to the local lesion and given over a 2 day period. A further

400 rads is given in 7 days irrespective of immediate results. Patients are separated into three groups: a) perianal or perineal lesions, b) axillary lesions, c) other sites. All patients go down to the Radiotherapy Dept. and allocations to radiotherapy are not communicated to the clinical staff until the patients trial has ended.

Major Findings:

Thirteen patients have been entered into this study, seven patients have received local radiotherapy and all lesions completely regressed by day 10. Six patients did not receive radiotherapy and three had complete resolution of lesions by day 16, one partial resolution after 3 weeks and two lesions improved but did not show further regression by day 21. No patient in either group has suffered from disseminated infection as a result of these local lesions. Although these numbers are too small as yet to come to any meaningful conclusions, it appears that meticulous attention to these lesions is of considerable importance in preventing disseminated infection. Localized radiotherapy appears to speed the rate of resolution but it is not apparent that this addition to local therapy is of greater value in preventing disseminated infection.

Significance to Cancer Research:

Overwhelming infection remains the principal cause of death in patients with acute leukemia and further methods to prevent and treat such infections are required. Patients with acute leukemia have reduced body defenses to cope with local infections and a significant mortality has been associated with dissemination from these sites in the past. The early results reported here have promise in preventing disseminated infection and the use of local radiotherapy appears to be of further benefit in speeding the healing of such lesions.

Proposed Course:

Continue present studies.

Honors and Awards:

None

Publications:

None

Serial No.: NCI-6915
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Staging Laparotomy and Combined Modality Therapy in Stage IA and IIA Lymphocyte Predominant, Nodular Sclerosing, and Mixed Cellularity Hodgkin's Disease

Previous Serial Number: None

Principal Investigators: Michael J. O'Connell, M.D., Peter H. Wiernik, M.D.

Other Investigators: Kirkland C. Brace, M.D., Roger Byhardt, M.D.

Cooperating Units: Radiotherapy Service, BCRC: Surgery Service, USPHS Hospital

Man Years:

Total: 1/2
Professional: 1/2
Other: 0

Objectives:

1. To determine whether modification of radiation therapy ports on the basis of staging laparotomy findings will significantly improve survival in patients with clinically localized Hodgkin's disease, as compared to radiation therapy administered without staging laparotomy.
2. To determine whether the addition of adjuvant chemotherapy to limited field radiation therapy will result in improved survival compared to limited field radiation alone or extended field radiation alone in patients with clinically localized disease who have undergone staging laparotomy.

Methods Employed:

Patients with newly diagnosed Hodgkin's disease undergo complete clinical evaluation including history, physical examination, serum chemistries, and lymphangiography. At this point patients are randomized to receive extended field radiation therapy alone, or alternatively to undergo staging laparotomy with subsequent therapy modified according to findings at surgery. Patients who undergo laparotomy are randomized to receive on of the 3 following regimens: 1) limited field radiation therapy alone, 2) extended field radiation alone, or 3) limited field radiation therapy followed by chemotherapy using nitrogen mustard, vincristine, prednisone and procarbazine.

Major Findings:

To date, only 5 patients have been entered into the study, and therefore, it is too early to make any assissment of the relative value of the various approaches outlined in this protocol.

Significance to Cancer Research:

Although staging laparotomy has been demonstrated to be of considerable benefit to many patients with Hodgkin's disease, there still exists controversy as to its value in patients with early localized Hodgkin's disease. This study should allow direct comparison of complication rates and overall survival in patients treated with radiation alone who have undergone staging laparotomy as opposed to patients treated with radiation alone who have not undergone staging laparotomy in a prospectively randomized clinical trial. In addition, the possible benefits of adjuvant chemotherapy and/or extended field radiation as opposed to involved field radiation should be evaluable, and may allow selection of the most effective and least toxic treatment regimen for patients with this potentially curable disorder.

Proposed Course:

Continue Investigation.

Honors and Awards: None

Publications: None

Presentations: None

Serial No.: NCI-6916
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Pilot Protocol for the Treatment of Patients with Stage III and IV Diffuse Histiocytic Lymphoma

Previous Serial No.: None

Principal Investigators: Michael J. O'Connell, M.D., Peter H. Wiernik, M.D.

Other Investigators: Viola Young, Ph.D.
Stephen Schimpff, M.D.
John Levi, M.D.
Charles Schiffer, M.D.

Cooperating Units: Section of Microbiology, BCRC

Man Years:

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives:

To determine whether high dose intensive combination chemotherpay of patients with disseminated diffuse histiocytic lymphomas, administered in laminar airflow isolation units to protect against infection, will result in an improved remission rate and prolonged survival.

Methods Employed:

Patients with biopsy proven malignant lymphoma of the diffuse histiocytic variety who have clinical stage III or stage IV disease are placed in reverse isolation in a laminar air-flow unit and given prophylactic non-absorbable oral antibiotics and treated with doses of chemotherapeutic agents designed to produce significant myelosuppression in an attempt to provide better control of this devastating disease than is possible with conventional doses of the same drugs. The agents employed in this study are cytoxan, adriamycin, vincristine, and prednisone.

Major Findings:

Only one patient has been entered on the study to date. Excellent tolerance to the high doses of chemotherapeutic agents employed, the stress of reverse

Major Findings: (Cont'd)

isolation, and the administration of the oral antibiotics was observed in this single patient. Essentially complete regression of her extensive nodal disease was noted, although followup has been too brief to determine whether the length of her remission will be longer than that usually seen in this disorder. Further patients are needed to more adequately evaluate this approach.

Significance to Cancer Research:

Disseminated diffuse histiocytic lymphoma is the most malignant variety of non-Hodgkin's lymphomas, with a median survival of less than one year from the time of diagnosis. Conventional chemotherapeutic regimens, although frequently producing some disease regression, are commonly ineffective in producing lasting disease control. Thus, if the infectious complications which are associated with severe bone marrow depression from chemotherapy can be avoided by the use of laminar air-flow units and prophylactic oral non-absorbable antibiotics, it may be possible to administer much higher doses of chemotherapy and thereby effect a more useful and long lasting remission of the disease.

Proposed Course:

Continue investigation.

Honors and Awards: None

Publications: None

Presentations: None

Serial No.: NCI-6917
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cyclophosphamide, Adriamycin, Vincristine, and Prednisone vs. Cyclophosphamide, Vincristine, and Prednisone for Disseminated Non-Hodgkin's Lymphoma

Previous Serial Number: None

Principal Investigators: Michael J. O'Connell, Peter H. Wiernik, M.D.

Other Investigators: John Levi, M.D.
Charles Schiffer, M.D.
Stephen Schimpff, M.D.

Cooperating Units: None

Man Years:

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives:

1. To determine whether the addition of adriamycin to cyclophosphamide, vincristine, and prednisone will improve remission induction rates in patients with disseminated non-Hodgkin's lymphomas.
2. To evaluate the efficacy of maintenance chemotherapy in prolonging both remission duration and survival in those patients who achieve complete remission following induction therapy.

Methods Employed:

Patients who are found to have stage III or stage IV non-Hodgkin's lymphomas on the basis of clinical staging procedures are randomly assigned to receive therapy with either cyclophosphamide, adriamycin, vincristine, and prednisone or cyclophosphamide, vincristine, and prednisone without adriamycin for induction therapy. Patients who achieve complete remission then undergo consolidation therapy with CCNU, bleomycin, and prednisone. A second randomization is then carried out with half the patients assigned to no further therapy and the remainder randomized to receive intermittent high dose cytoxan monthly intravenously until relapse occurs.

Major Findings:

It has been determined on the basis of a preliminary pilot study that cyclophosphamide, adriamycin, vincristine and prednisone can be given in combination with acceptable toxicity in the following dosage schedule: cyclophosphamide -- 1000mg/M^2 intravenously on day 1, adriamycin -- 45mg/M^2 intravenously on day 1, vincristine -- 1.4 mg/M^2 days 1 and 8, and prednisone -- 40 mg/M^2 daily on days 1 through 10. These agents are administered in 21 day cycles. Since only a few patients have been entered on the randomized study, it is too early to indicate whether the addition of adriamycin will be of benefit.

Proposed Course:

Continue investigation.

Significance to Cancer Research:

Conventional chemotherapy regimens have produced at best a 50% complete resopnse rate in the non-Hodgkin's lymphomas, with the majority of patients ultimately relapsing and dying of progressive disease for complications of its therapy. Furthermore, an attempt to provide curative therapy, modeled after the approach which has been successful in acute lymphatic leukemia, employing intensive consolidation therapy is being studied. This study is designed to determine whether these results can be improved by adding the new anthracycline antibiotic adriamycin to the induction therapy. Finally, it may be possible to determine the need for long term maintenance therapy in patients who achieve remission through the randomized scheme employed in this study.

Honors and Awards: None

Publications: None

Presentations: None

Serial No.: NCI-6918
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Streptozotocin, Bleomycin, CCNU and Adriamycin for the Treatment of Advanced, Refractory Hodgkin's Disease

Previous Serial Number: None

Principal Investigator: Michael J. O'Connell, M.D.

Other Investigators: Peter H. Wiernik, M.D.
John A. Levi, M.D.

Cooperating Units: None

Man Years:

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

To determine the clinical usefulness of a new combination chemotherapy regimen for advanced Hodgkin's disease which is no longer responsive to conventional chemotherapy approaches.

Methods Employed:

Patients with advanced biopsy proven Hodgkin's disease who have failed conventional chemotherapy approaches are treated with a combination of streptozotocin 0.5 gm/M^2 daily for 5 days, CCNU 100 mg/M^2 on day 1, bleomycin 15mg/M^2 intramuscularly on days 1 and 8, and adriamycin 45 mg/M^2 on day 1 with cycles repeated each 28 days if blood counts permit.

Major Findings:

Only 2 patients have been treated with regimen to date. Toxicity has been acceptible and objective tumor regression has been seen in both patients.

Proposed Course:

Continue investigation.

Serial No.: NCI-6918

Significance to Cancer Research:

Although currently available combination chemotherapy regimens, such as MOPP, have been shown to produce complete remissions in approximately 3/4 of patients treated, there is still a sizable number of patients with previously untreated Hodgkin's disease who do not achieve remission utilizing these drugs. In addition, patients treated with radiation therapy for local disease occasionally relapse and a proportion of these patients prove refractory to standard chemotherapy approaches. Therefore, a need for an effective alternative regimen exists. Streptozotocin has been shown in preliminary stage II studies to have definite activity against refractory Hodgkin's disease and is an attractive agent for combination chemotherapy since it has very mild bone marrow suppressive characteristics. Adriamycin, likewise, it a new agent which has been shown to have activity against Hodgkin's disease whose structure and mechanism of action is quite different than the agents commonly employed. Bleomycin has produced objective regressions in otherwise resistant Hodgkin's Disease and it too has little bone marrow suppressive characteristics. CCNU has been shown to have significant activity in patients refractory to alkylating agents and vinca alkaloids. Thus, it is hoped that the combination of these 4 different chemotherapeutic agents with different mechanisms of action and different toxicities will be of benefit to patients with Hodgkin's disease who are not responsive to standard chemotherapy regimens.

Honors and Awards: None

Publications: None

Presentations: None

Serial No.: NCI-6919
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Initial Drug Therapy for Metastatic Renal Cell Carcinoma

Previous Serial Number: None

Principal Investigators: John C. Ruckdeschel, M.D.

Other Investigators: Peter H. Wiernik, M.D.
Stephen C. Schimpff, M.D.

Cooperating Units: Department of Hematology, Veterans' Administration Hospital, Seattle, Washington (Dr. R. Adamson)

Man Years:

Total: 1
Professional: 1
Other: 0

Project Description:

Objectives:

Renal cell carcinoma is a solid tumor of notorious resistance to chemotherapy and radiation therapy. Although surgery may be curative in some cases, the overwhelming percentage of cases proceed to metastatic disease and death within two years of diagnosis. Previous chemotherapy studies have been, almost without exception, inadequate. The few studies that have had adequate patients and adequate controls have indicated either no activity for such drugs as adriamycin or minimal activity for such drugs as provera. One agent that has received some notice in terms of treatment of renal cell carcinoma in CCNU; a response rate on the order of 10-15% has been seen in one study with this agent, and a similar response rate for vinblastine was noted in another study. Dr. James Holland recently reviewed material on a derivative of thio-TEPA that underwent evaluation in the Soviet Union for use in renal cell carcinoma and exhibited a response rate on the order of 50%. Previous work in this country with thio-TEPA was limited to a few case reports a number of years ago and the drug has never been thoroughly evaluated here. The major objective of this study will be to examine the efficacy of vinblastine, CCNU, or thio-TEPA in standard doses for the initial management of metastatic renal cell carcinoma. The second objective of this study will be in cooperation with the Veterans' Administration Hospital Department of Hematology, Seattle, Washington in cooperation with other investigators in that laboratory at the University of

Objectives (Cont'd):
Washington Medical School. Specimens of malignant tissue will be sent to Seattle for attempted tissue culture and extraction of various erythropoietin and erythropoietin-like products. An attempt to understand the various factors operative in anemia and polycythemia seen with renal cell carcinoma will be investigated.

Major Findings:

None to date. Protocol just activated.

Proposed Course:

Continue investigation.

Honors and Awards: None.

Publications: None to date.

Presentations: None to date.

Serial No.: NCI-6920
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: 5 Azactidine vs. Guanazole for the Therapy of Previously Treated Adult Acute Non-Lymphocytic Leukemia

Previous Serial No: None

Principal Investigators: Michael J. O'Connell, M.D., Peter H. Wiernik, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives:

To determine the relative efficacy of 2 new experimental chemotherapeutic agents, 5-Azacytidine and Guanazole, in the treatment in advanced adult acute non-lymphocytic leukemia which is no longer responsive to conventional chemotherapeutic agents.

Major Findings:

Eighteen patients have been entered on the study, 8 treated with Azacytidine and 10 with Guanazole. Seven patients given Azacytidine and 9 patients treated with Guanazole are currently evaluable. Three patients (43%) treated with Azacytidine achieved complete remission, and 1 patient (11%) treated with Guanazole achieved partial remission. Azacytidine appears to be a highly active agent in ANLL. Unmaintained responses are short (1 to 3 months). Moderate muscular weakness is seen in most patients after Azacytidine treatment and pulmonary infiltrates probably secondary to Guanazole were seen in 1 patient.

Proposed Course:

Continue investigation.

Serial No.: NCI-6920

Honors and Awards: None

Publications: None

Presentations: None

Serial No.: NCI 6921
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Adriamycin Therapy for Advanced Mycosis Fungoides

Previous Serial Number: none

Principal Investigators: John A. Levi, M.D., Peter H. Wiernik, M.D.

Other Investigators: Michael O'Connell, M.D., Roger Byhardt, M.D., Kirkland Brace, M.D.

Cooperating Units: Section of Radiotherapy

Man Years:

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives:

To determine the efficacy of adriamycin therapy for the treatment of advanced mycosis fungoides.

Methods Employed:

Adriamycin was given to patients with a biopsy-proven diagnosis of mycosis fungoides who were admitted to the BCRC. All patients had received previous therapy and were now refractory. Disease status was advanced, extensive skin disease, lymphadenopathy was present in all cases and visceral disease was shown to be present in two. Adriamycin was administered in a dose of 60 mg/m^2 IV every 3 weeks and doses modified if any liver dysfunction or significant myelosuppression occurred. A minimum of 3 courses were given and if complete response ensued, a further 2 courses beyond complete response given. Maintenance therapy consists of methotrexate 15 mg/m^2 IM twice weekly and cytoxan 750 mg/m^2 IV every 3 weeks.

Major Findings:

Six patients have been entered into this study and 5 of these are evaluable at this point. There has been one complete response, 3 partial responses and one failure. Median response has not yet been determined but it appears

Major Findings (cont'd):

that adriamycin is effective in the treatment of advanced mycosis fungoides. Toxicity has been mild and only a moderate degree of myelosuppression encountered.

Significance to Cancer Research:

Mycosis fungoides is an unusual form of lymphoma which often has a slow course early in its stages of development. However, once the disease is advanced, there is often rapid progression and no form of chemotherapy used to this time has improved survival. Adriamycin appears to be an effective agent and if intensive therapy with this drug can produce a number of responses and prolong duration of response and survival time, then further studies with this agent alone and in combination with others may improve the outlook for patients with this distressing disease.

Proposed Course: Continue present studies.

Honors and Awards: None

Publications: See NCI #6279

Presentations:

Levi JA: A more aggressive approach to the treatment of mycosis fungoides. Meeting of the American Society of Dermatologists, Maryland Division, University of Maryland, November 15, 1973.

Serial No.: NCI 6922
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Epidemiologic Investigations of Possible Social Clustering Among Patients with Leukemia and Lymphoma

Previous Serial Number: None

Principal Investigator: Stephen C. Schimpff, M.D.

Other Investigators: Carol R. Schimpff, Dorah Brager, Peter H. Wiernik, M.D.

Cooperating Units: George Comstock, M.D., Johns Hopkins School of Epidemiology and Public Health: Frances Shaver, RRL, West Virginia Cancer Control Bureau, Charleston, West Virginia

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives:

Both leukemias and lymphomas have variously been suggested as diseases possibly caused by an infectious agent. Recent published observations have suggested that some patients with Hodgkin's disease may have been close personal contacts with others who subsequently developed the disease. The BCRC investigation was instituted to assess the possible relevance of inter-personal contact in the epidemiology of both leukemia and lymphoma.

Major Findings:

Over the past three years, five distinct social clusters of lymphoma and leukemia have been detected by analysis of the personal interactions of 75 patients. Detailed personal interview techniques include 1) case-to-case followup beginning with an index case in each area and 2) queries to available patients or surviving relatives following review of tumor registry data. In these five areas, 23, 8, 12, 15, and 17 patients, respectively, have been linked together via their past close social contacts. Each of the five clusters was detected in semi-rural regions with stable popula-

Major Findings (cont'd):

tions where personal associations dating back over many years could be traced with reasonable ease and accuracy. These five areas were investigated because of our suspicion of clustering. Therefore, to eliminate a possible bias of preselection, 20 clinic patients with Hodgkin's disease were randomly chosen for interviews; 6 (5 from rural areas) indicated that they had one or more close personal contacts who have leukemia or lymphoma. These findings are consistent with the possibility that these diseases may be caused by horizontally transmissible agents.

Proposed Course: Continue study.

Significance to Cancer Research:

Such investigations may assist in defining some aspects in the causation of specific cancers.

Honors and Awards: None

Publications:

1. Schimpff SC, Schimpff CR, Wiernik PH: Five clusters of leukemia and lymphoma interlinked by prior social contact. Clinical Research, 22, 1974.

Presentations:

Schimpff SC: Epidemiologic considerations in the development of leukemias and lymphomas - five clusters interlinked by prior social contact. Infectious Disease Research Conference - University of Maryland, January, 1974.

Serial No.: NCI-6024
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Development of a Neurosurgical Service with Primary Interest in the Treatment of Brain Tumors at the BCRC, NCI

Previous Serial Number: Same.

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Howard D. Weiss, M.D.
Marshall A. Levine, M.D.
Philip H. Gutin, M.D.

Cooperating Units: Medical Service, BCRC, NCI
Department of Surgery, US PHS Hospital, Baltimore, Md.

Man Years:

Total:	1.5
Professional:	1.5
Other:	0

Project Description:

Objectives:

This ongoing project is designed to develop an effective research neurosurgical service to evaluate various modalities of treatment for intracranial neoplasms. Pilot studies are performed to evaluate the efficacy of drugs in relation to Phase I-II and neuropharmacologic studies. Neurosurgical consultation, neurodiagnostic, and therapeutic services are provided for the US PHS Hospital, Baltimore. Consultation and supervision of NCI projects related to the treatment of malignancy within the intracranial space are made available. Interdisciplinary studies of blood brain barrier, pharmacology and cellular membrane permeabilities are undertaken.

Methods Employed:

Patients with diagnosed intracranial neoplasm are referred to the BCRC, Section of Neurological Surgery, for evaluation and chemotherapy from both the general medical community, as well as the PHS system. Pharmacologic evaluation of chemotherapeutic agents, intracranial markers, and specific and non-specific humoral indicators of malignancy may be carried out as part of the operative procedure in patients undergoing surgery at the BCRC. Complete care on an out- and inpatient basis, as well as utilization of the

Methods Employed (cont'd)

intensive care unit and blood component therapy services are available. Due to the potential toxicity of drugs employed, close monitoring of patients is a necessity. Studies utilizing Epipodophyllotoxin (PTG, VM-26) and a newly developed intravenous preparation of IV Methyl CCNU have been completed, and combination chemotherapy using these agents is being investigated. This service has also participated in the Brain Tumor Study Group.

Frequent consultations and neurologic evaluation of BCRC patients are provided the medical staff to assist in the general medical oncology program. A particular interest in CNS leukemia, as well as metastasis of other tumors, is pursued. Phase I studies of intrathecal thiotepa, the first intrathecal alkylating agent used against meningeal neoplasms, has recently been completed. CSF clearance studies of inert substances has been carried out.

In consultation with the Departments of Medicine, Surgery and Neurology of US PHS Hospital, Baltimore, neurosurgical evaluation, neurodiagnostic procedures and surgical intervention for general neurosurgical disease are provided. Consultations on difficult diagnostic problems are undertaken. Through the Director, surgical and parasurgical communications are maintained. Formal and informal consultations are provided to the greater Baltimore medical community in relation to brain tumor therapy. Informal discussions, telephone consultations, and formal lectures are given.

Major Findings:

During the fiscal year, there were 45 neurosurgical admissions, 113 formal consultations, and 525 out-patient visits. A Phase II study of intravenous Methyl CCNU has been completed and demonstrated 5 satisfactory responses out of 16 evaluable patients with primary malignant brain tumor. A similar Phase II study of PTG has provided a remission rate and duration similar to that obtained with the nitrosoureas but with less marrow suppressant toxicity. A Phase I toxicology and pharmacologic study of a newly developed intravenous preparation of CCNU has been undertaken. Phase I studies of intrathecal thiotepa have demonstrated no serious neurotoxicity or myelosuppression at doses from 1-10 mg/m^2 twice weekly, with complete resolution of meningeal leukemia occurring with doses as low as 2 mg/m^2.

The lack of systematized criteria and truly measurable disease in patients with primary brain neoplasm has long thwarted therapeutic evaluation. A CNS grading system based upon symptoms and function as well as neurologic examination has been developed to provide a numerical grade for the patient during the course of his disease. Factors other than therapeutic response which may affect the staging system have been consistently dealt with. The CNS disease staging system is continuing to be evaluated.

Consultation services within the hospital, the city of Baltimore, nationally and internationally have been provided. Lectures, papers and discussions have been undertaken within the neuroscience community in order to effectively bring out the Division of Cancer Treatment's programs.

Significance to Biomedical Research and Program of the Institute:

The incidence of primary central nervous system neoplasms exceeds that of Hodgkin's disease. With increasingly successful therapy of cancer elsewhere in the body, cerebral metastasis is becoming a more important obstruction to conquest of these diseases. CNS leukemia illustrates this problem well as it may be the sole sanctuary of the disease which prevents its cure. Development of a study unit to evaluate the efficacy, toxicity, and neuropharmacology of drugs used to treat these disease processes is essential to future control of cancer. Development of therapeutic and toxicologic data related to the treatment of primary brain tumor from the utilization of the CNS staging system provides an objective scale for the more reliable analysis of data.

Proposed Course:

This service will continue to admit, diagnose and treat operatively and chemotherapeutically and radiotherapeutically patients with intracranial malignancy. Phase I studies of new chemotherapeutic agents with appropriate pharmacokinetic characteristics for successful brain tumor chemotherapy will be undertaken. Phase II studies of intravenous Methyl CCNU in combination with PTG for brain tumors and for sarcomas will proceed, as well as investigation of intravenous CCNU. Continued participation in the Brain Tumor Study Group will be undertaken and consultation services, as described above, will also continue.

Honors and Awards: None.

Publications:

Walker, M.D.: Physiological Barriers to Pharmacologic Efficacy. Fifth Int. Congr. Pharm. 3: 354-364 (Karger, Basil, 1973).

Band, P.R., Holland, J.F., Weil, B.J., Walker, M.D., and Rall, D.P.: Treatment of Central Nervous System Leukemia with Intrathecal Cytosine Arabinoside. Cancer 32: 744-748 (1973).

Weiss, H.D.: Methotrexate Neurotoxicity. New Engl. J. Med. 290: 56-57 (1974).

Presentations:

American Academy of Neurology, Boston, Mass.: "Principles of CNS Tumor Therapy-Principles of Surgery and Principles of Chemotherapy, August 25, 1973.

Children's Cancer Research Foundation, Boston, Mass.: "Why Treat Brain Tumor?" May 15, 1973.

NINDS Brain Tumor Seminar, Bethesda, Md.: "Concepts in the Treatment of Malignant Glioma." September 14, 1973.

Fifth International Congress of Neurological Surgery, Tokyo, Japan: "The Treatment of Malignant Glioma in Controlled, Prospective and Randomized Trials." October 10, 1973.

University of Maryland School of Medicine Program of Continuing Medical Education: "Recent Advances in the Treatment of Intracranial Neoplasm." November 8, 1973.

George Washington University Hospital Neurosurgery-Neurology Conference, Washington, D.C.: "Clinical Presentation of Malignant Brain Tumor." January 11, 1973.

Medical Science Lectures of the Health Record Administration Program, USPHS Hospital: "Surgery of Central Nervous System." January 15, 1973.

St. Agnes Hospital Department of Surgery Lectures, Baltimore, Md.: "Chemotherapy of Tumors of the Central Nervous System." January 16, 1974.

Interurban Neurosurgical Society, Chicago, Ill.: "Combined Treatment of Central Nervous System Tumors." February 2, 1974.

Department of Nuclear Medicine Seminar of the University of Maryland Hospital, Baltimore, Maryland: "Advances in the Treatment of Brain Tumors."

American Association of Neurological Surgeons Meeting, St. Louis, Missouri: "Transplantation of Nervous System Tumors in Diffusion Chambers" by Dr. F.K. Conley. Discussor and moderator on April 24, 1974.

American Academy of Neurology Meeting, San Francisco, Calif.: "Chemotherapy of Malignant Glioma with BCNU & Vincristine Sulfate" by Dr. W. Shapiro. Discussor on April 27, 1974.

Serial No.: NCI-6202
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Murine Intracerebral Melanoma Study

Previous Serial Number: Same

Principal Investigator: Donald L. Bowie, B.S.

Other Investigators: Michael D. Walker, M.D.
John Hilton, D.Phil.

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0
Other:	0.3

Project Description:

Objectives:

The major objectives of this project are to continue the study of the efficacy of MeCCNU against the murine intracerebral melanoma, and the interaction of various drugs with this chemotherapeutic agent.

Methods Employed:

B-16 melanoma of the Jackson Laboratory, Bar Harbor, Maine, has been employed for serial transplant. Varying doses of IV-MeCCNU have been used. Before MeCCNU was given animals were pretreated with saline, phenobarbital, SKF-525 A, decadron and dilantin. Tumored mice were treated with varying doses of MeCCNU for 24 hours and then given tritiated thymidine for 1 hour.

Major Findings:

Phenobarbital reduced toxicity of the LD_{10}, 50, and 90. MeCCNU (30 mg/kg) increased the lifespan of tumor-bearing mice by 70%. Pretreatment with phenobarbital appeared to decrease the efficacy of MeCCNU (30 mg/kg); the increase in lifespan being only 30%. There occurred a slight decrease in lifespan of animals pretreated with decadron, dilantin, and thorazine. SKF-525A had no effect on the lifespan of tumor-bearing animals, but it did

Major Findings (cont'd)

increase the toxicity of MeCCNU from the LD_{10} to the LD_{50} and the LD_{50} to the LD_{90}.

In DNA studies, tritiated thymidine incorporation into DNA was inhibited 50% at a minimum dose of 5 mg/kg MeCCNU.

Proposed Course:

Further studies of the effect of phenobarbital and SKF-525A on the efficacy of MeCCNU will be undertaken at the cellular and subcellular levels of the tumor. Further studies on time course of and dose response levels of inhibition of DNA synthesis will also be undertaken.

Honors and Awards: None

Publications: None

Presentations: None

Serial No.: NCI-6204
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Toxicity of Intracerebral Chemotherapy in Monkeys

Previous Serial Number: Same

Principal Investigators: Michael D. Walker, M.D.
Howard D. Weiss, M.D.
Archie Bleyer

Other Investigators: Dan Dalgard, D.V.M.

Cooperating Units: Hazelton Laboratories, Falls Church, Virginia

Man Years:

Total:	.75
Professional:	.25
Other:	.5

Project Description:

Objectives:

With the increase in meningeal leukemia and the interest in neoplasms within the central nervous system, the use of intrathecal chemotherapy is expected to increase. In this ongoing project, a relatively standardized technique for evaluating intrathecal chemotherapy in monkeys is being employed.

Methods Employed:

Intracisternal installation of carefully chosen drugs are first performed to determine acute immediate toxicity. From this information dose levels for chronic studies simulating the dose schedules used in man are developed. Evaluation of the activity in indices, hematologic parameters and cerebral spinal fluid are made. Histopathologic review is performed on the upper cervical cord, brain stem and brain.

Major Findings:

Previously the intrathecal toxicity of thiotepa was defined at the toxic dose low at 6 mg/m^2. Histopathologic evaluation of the brain and cervical spinal cord of animals dosed in this and higher (12 mg/m^2) ranges have failed to yield neuropathologic changes. Dose seeking studies on intrathecal adriamycin have indicated extreme sensitivity with delayed neurotoxicity at doses as low as

1/10 mg/m^2. The precise mechanisms is yet to be elucidated, but may relate to binding characteristics of the anthracycline antibiotics. CSF pharmacodynamics studies with methotrexate have been undertaken in order to more clearly delineate the neurotoxicity and convulsive behavior encountered with this substance. Phencyclodine hydrochloride, commonly used tranquilizers for monkeys, appears to have some synergistic effect with methotrexate and consequently investigations have been undertaken in the utilization of curare in small doses. This appears to be highly effective and reduces the neurotoxicity from methotrexate.

Significance to Biomedical Research and Program of the Institute:

Intrathecal chemotherapy for human neoplasms has been limited to methotrexate cytosine arabinoside and intrathecal thiotepa are currently being evaluated on Phase I. As the control of cancer in the periphery becomes greater, the chances of developing CNS metastasis similarly increase. Therefore, developing appropriate toxicologic information concerning drugs placed in the CSF pathways will provide necessary information for expanding intrathecal drug usage.

Proposed Course:

Additional drugs will be added to the study this year as well as a more careful evaluation of previously investigated drugs. In conjunction with the Laboratory of Pharmacology, pharmacokinetic distribution studies will be undertaken in the monkey so as to more clearly understand the effects seen in this animal. Further work utilizing a curarized animal must be followed.

Honors and Awards: None

Publications: None

Presentations:

American Association for Cancer Research, Houston, March 28, 1974, "Preclinical and Phase-I Clinical Studies of Intrathecal N,N'N"-Triethylenephosphoramide (Thiotepa NSC-6396)", H. D. Weiss, M. D. Walker, P. H. Wiernik, and D. Dalgard.

Serial No.: NCI-6268
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Radio-labeled Distribution of Methyl CCNU in Dogs

Previous Serial Number: Same

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Donald L. Bowie, B.S.
John Hilton, D.Phil.

Cooperating Units: Laboratory of Toxicology, DCT, NCI

Man Years:

Total:	0.3
Professional:	0.2
Other:	0

Project Description:

Objectives:

The emphasis of this project has shifted toward the determination of the pharmacological consequences of the early metabolism of Methyl CCNU and CCNU. Using the dog, the metabolites can be obtained in amounts sufficient to attempt chemical characterization.

Methods Employed:

Plasma of animals receiving intravenous nitrosourea is extracted into organic solvent. The extracts are fractionated by thin layer and high speed liquid chromatography.

Major Findings:

Our results indicate the feasibility of this approach toward the isolation of milligram amounts of Methyl CCNU and CCNU metabolites.

Proposed Course:

To proceed toward the isolation and characterization of polar metabolites of Methyl CCNU and CCNU.

Serial No.: NCI-6268

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6282
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Brain Tumor Study Group

Previous Serial Number: Same

Principal Investigators: Michael D. Walker, M.D.
Thomas A. Strike, Ph.D.

Other Investigators: Mathilde Solowey, Ph.D.
Maris Udey
Members of the BTSG

Cooperating Units: Members of the BTSG

Man Years:

Total:	2.1
Professional:	1.1
Other:	1.0

Project Description:

Objectives:

The Brain Tumor Study Group was established in order to provide a rapid, scientific and controlled method of evaluating chemotherapy in the treatment of brain tumors utilizing Phase III type studies. The program has been broadened to include evaluation of radiation therapy as a modality alone and in combination with chemotherapy. Additionally, the group is also undertaking Phase II studies in order to make the selection of chemotherapeutic agents more meaningful.

Methods Employed:

Members of the Brain Tumor Study Group have established a cooperative ongoing continuous effort providing newly-operated upon patients for randomization to protocol studies. Final data analysis for a Phase III protocol evaluating BCNU and irradiation alone and in combination in a prospective, controlled and randomized study is underway. The current Phase III protocol is evaluating methyl CCNU alone and in combination with radiation, radiation alone, and BCNU in combination with radiation. This study is approximately half completed. In addition, the program has been broadened to include the investigation of four new drugs in a cross-over Phase II protocol design.

Methods Employed (cont'd)

Adriamycin, Dibromodulcitol, Procarbazine, and Streptozotocin are being evaluated in patients with recurrent malignant glioma in a paired, randomized study. Patients deemed failures to therapy with one of the aforenamed drugs are rerandomized to either a high tapering dose of corticosteroids, or a moderate prolonged course of corticosteroid. Administrative support is provided by the Central Office, and institutional support by the contract mechanisms. Meetings of the Brain Tumor Study Group are at quarterly intervals, and staff visits from the Central Office provide appropriate communication and exchange of information.

Major Findings:

The BCNU irradiation protocol has had 302 patients randomized into its four arms, of which over three quarters are considered within the valid study group. Final evaluation of the data is underway. However, an intermediate evaluation indicates that surgical therapy alone provides a median survival of 14 weeks, BCNU a median survival of 21 weeks, radiotherapy a median survival of 31 weeks, and BCNU plus irradiation a median survival of 41 weeks.

The BCNU/methyl CCNU/irradiation protocol is approximately half completed in its patient accrual and is making satisfactory progress.

The Phase II studies have accrued over 20 patients on each arm. However, the data is too early for analysis.

The BTSG held a multidisciplinary meeting in the fall in which scientific papers on neuropathology, brain scan techniques, and irradiation physics were presented. This meeting provided a major focus for interdisciplinary activities.

Significance to Biomedical Research and Program of the Institute:

These studies are the only prospective, controlled, randomized studies performed in the United States in the treatment of brain tumor. It provides specific detailed and timely information concerning the efficacy of various therapeutic modalities for the malignancy of the central nervous system.

Proposed Course:

A final analysis of the BCNU-irradiation study is being undertaken and preparation for presentation. The BCNU/methyl CCNU/irradiation protocol will continue to accrue patients and is expected to be completed within a year. From the four Phase II drugs currently under study, it is expected that one or two of these agents will be chosen for the next controlled, prospective, randomized Phase III study of the group.

The BTSG also intends to expand its activities and taken in several new members during the course of the year so as to provide more rapid information

Proposed Course (cont'd)

and accrual of patients.

Honors and Awards: None

Publications:

Walker, M.D.: Nitrosoureas in Central Nervous System Tumors. Cancer Chemother. Rep. Part 3, 4: 21-26, 1973.

Presentations:

Fifth International Congress of Neurological Surgery, Tokyo, October 7-13, 1973, "The Treatment of Malignant Glioma in Controlled, Prospective and Randomized Trials", M. D. Walker, M. Solowey and E. A. Gehan.

Serial No.: NCI-6283
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: An Evaluation of 4'Demethyl-Epipodophyllotoxin-β-D Thenylidene Glucoside (PTG in a Phase II Study Treating Primary Intracranial Neoplasms

Previous Serial Number: Same

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Howard D. Weiss, M.D.
Marshall A. Levine, M.D.
Philip H. Gutin, M.D.

Cooperating Units: Medical Service, BCRC, NCI

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives:

This Phase II study was designed to evaluate the efficacy of PTG in the treatment of primary intracranial neoplasms and provide further toxicologic information concerning multiple doses of this lipid soluble drug.

Methods Employed:

Patients harboring primary intracranial neoplasms were treated with PTG giving multiple courses each consisting of 100-130 mg/m^2 administered intravenously weekly for 6-8 weeks. Drug responses were measured by using a standardized central nervous system disease staging system as well as a lumbar puncture, brain scan, etc. Frequent hematologic and biochemical parameters were obtained.

Major Findings:

Twenty-three patients with primary intracranial neoplasms were treated with PTG, three were considered unevaluable because they died after receiving considerably less than 1 full course of drug. The twenty evaluable patients have received between 1 and 11 courses of drug. Objective neurologic

Major Findings (cont'd)

improvement was noted in 4 of 12 patients who had measurable disease at the outset of therapy. The progression-free interval among these 4 remission patients was 7+ months as of January 1, 1973. Among the 8 patients who are without measurable disease at the outset of therapy, the progression-free interval was 15+ months to January 1, 1973. Two of these latter patients progressed prior to January 1, 1973, and 2 have subsequently progressed after that date. Toxicity was mild although consistent anemia and mild leukopenia were observed. Clinically significant marrow toxicity was seen only in 3 patients all of whom had prior extensive chemotherapy.

Significance to Biomedical Research and Program of the Institute:

Analysis of results indicates PTG to have some efficacy in the treatment of primary intracranial neoplasms with minimal toxicity.

Proposed Course:

Study terminated - drug to be employed in combination with other chemotherapeutic agents.

Honors and Awards: None.

Publications:

Sklansky, B.D., Kaplan, R.S., Reyholds, A.S., Rosenblum, M.L., Walker, M.D.: 4'-Demethyl-Epipodophyllotoxin-β-D Thenylidene Glucoside in the Treatment of Primary Intracranial Neoplasms. Cancer 33: 460-476 (1974).

Presentations: None.

Serial No.: NCI-6286
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Methyl CCNU: Phase I Toxicologic and Neuropharmacologic Evaluation and Phase II Therapeutic Study

Previous Serial Number: Same

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Peter H. Wiernik, M.D.
Howard D. Weiss, M.D.
Marshall A. Levine, M.D.
Donald L. Bowie, B.S.
John Hilton, D. Phil.
Philip H. Gutin, M.D.

Cooperating Units: Medical Service, BCRC, NCI

Man Years:

Total:	0.2
Professional:	0.2
Others:	0

Project Description:

Objectives:

Pharmacologic investigation of this nitrosourea and a Phase II study of its therapeutic efficacy in malignant glioma and tumor metastatic to the central nervous system have been completed.

Methods Employed:

Patients with progressive central nervous system symptoms, after appropriate surgery, radiotherapy or chemotherapy, were referred to the Neurosurgical Section, Baltimore Cancer Research Center, NCI, for treatment with newer chemotherapeutic agents capable of crossing the blood-brain barrier. A number of patients of the Medical Service, BCRC, NCI, with metastatic tumor involvement of the central nervous system were also referred for study.

Methyl CCNU is administered by the oral route in optimal doses as previously established in the Phase I portion of this study. These are 290 mg/m^2 in previously untreated patients, and 220 mg/m^2 or lower in those patients with bone marrow compromise from previous therapy, tumor infiltration, etc. Careful

Methods Employed (cont'd)

Methyl CCNU is administered by the oral route in optimal doses as previously established in the Phase I portion of this study. These are 290 mg/m^2 in previously untreated patients, and 220 mg/m^2 or lower in those patients with bone marrow compromise from previous therapy, tumor infiltration, etc. Careful monitoring of hematologic and toxicologic parameters were obtained and the patients were supported with blood products, platelets and white cells when required. Select patients received, along with their therapeutic dose of methyl CCNU, radiolabelled tracer doses for pharmacologic study. Blood, urine, cerebrospinal fluid and normal and abnormal tissues are analyzed for radioactivity, and drug and metabolite content.

Major Findings:

The dose of 290 mg/m^2 in previously untreated patients was confirmed and further pharmacologic studies revealed no important variations in the pharmacology as previously described.

Thirty-two patients were therapeutically evaluable. Twenty-seven of these patients had intracranial tumors, of which 23 were primary and 4 were metastatic neoplasms. Of the 23 patients with primary brain tumors, 19 had adequate trials with the drug (at least one full course at what has been established as adequate dosage). Sixteen of these patients had malignant gliomas, 3 of whom were without measurable disease at the intiation of chemotherapy, and all 3 have remained well from 18 weeks to more than 1 year after beginning MeCCNU. Thirteen patients had measurable neurologic dysfunction, and 3 of these achieved a partial remission lasting from 16 to more than 60 weeks. Of the 19 patients with primary brain tumors, 8 have had satisfactory responses to MeCCNU lasting 16 to 60+ weeks. Of the 5 patients who did not have intracranial tumors, there was only 1 short-lived response in a patient with a reticulum cell sarcoma.

Significance to Biomedical Research and Program of the Institute:

The nitrosoureas are among the most active of the antineoplastic compounds available. Preliminary studies would indicate that the level of therapeutic response to oral MeCCNU is equivalent to that seen with BCNU. Determination of tolerable dose, toxicity, pharmacologic characteristics, and predictive parameters will allow more intelligent and objectively founded choice of therapeutic regimen and delivery of that regimen in the future.

Proposed Course: The Phase II study of oral MeCCNU was completed this year and the Phase III BTSG study employing this drug will be followed.

Honors and Awards: None.

Publications: None.

Presentations: None.

Serial No.: NCI-6904
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1974

Project Title: Studies on the Anti-Tumor Activity of Methyl CCNU and Related Compounds at the Subcellular Level

Previous Serial Number: Same

Principal Investigator: John Hilton, D. Phil.

Other Investigators: Michael D. Walker, M.D.
Donald L. Bowie, B.S.
John Hiemenz, Summer Student

Cooperating Units: None

Man Years:

Total:	0.7
Professional:	0.2
Others:	0.5

Project Description:

Objectives:

To determine the site of action of methyl CCNU and related compounds as tumor growth inhibitors. This will allow a judicious choice of co-inhibitors used in combination chemotherapy.

Methods Employed:

The nitrosourea induced rat glioma used in these studies is maintained in culture. Cell suspensions are introduced stereotactically into the parietal white matter or subcutaneously in the flank of male rats. Methyl CCNU and related compounds, suspended in a lipid emulsion, are administered via the tail vein.

Deoxyribonucleoside triphosphate pool sizes were determined in acid extracts of glioma cells in culture and in extracts of "freeze-clamped" tumor and regenerating liver tissue. The methods of Solter and Handschumacher (BBA 174, 585, 1969) and Lindberg and Skoog (Anal. Biochem. 34, 152, 1969) were used.

Glioma cell extracts were fractionated by centrifugation in a sucrose density gradient. Fractions of the gradient were analyzed for "nuclear" and "cytoplasmic" DNA polymerase activities.

Major Findings:

Studies on the antineoplastic activity of methyl CCNU are in progress using a nitrosourea induced, transplantable rat glioma as the target tissue. The tumor, implanted either intracerebrally or subcutaneously, is susceptible to growth inhibition by MeCCNU. DNA synthesis, measured by radioactive thymidine incorporation, is the most sensitive macromolecular synthetic process affected by methyl CCNU. The onset of inhibition of DNA synthesis is rapid (3 hours after methyl CCNU) and inhibition lasts for about 12 days after treatment. Similar inhibition of thymidine incorporation into DNA by methyl CCNU is observed in the intracerebrally implanted or flanked implanted glioma.

The immediate precursor of DNA thymidine is thymidine triphosphate (TTP). A decrease in the specific radioactivity of the pool of TTP following the administration of labelled thymidine could account for the observed effect of methyl CCNU on thymidine incorporation into DNA. No abnormality in the pool size or specific activity of TTP commensurate with the inhibition of thymidine incorporation into DNA has been observed in tumor tissue from methyl CCNU treated animals.

This project has proceeded to investigate the sensitivity of glioma DNA polymerases after treatment of tumor bearing animals with IV methyl CCNU. The not unexpected *in vitro* sensitivity of E. coli DNA polymerase I to BCNU which has been reported in the literature was not considered relevant to the antitumor activity of the nitrosoureas *in vivo*.

Treatment of tumor bearing animals with IV methyl CCNU did not significantly alter the cytoplasmic DNA polymerase activity. In contrast, the nuclear DNA polymerase activity was halved in tumors from animals receiving methyl CCNU compared to controls. The earliest significant change in nuclear polymerase activity occurred at about 12 hours after methyl CCNU administration.

In an attempt to determine the nature of the effect of methyl CCNU on nuclear DNA polymerase activity some preliminary experiments were performed using the regenerating rat liver as an advantageous model. While inhibition by methyl CCNU of the incoporation of radioactivity from ^{3}H thymidine could be demonstrated readily, on analysis of DNA precursor pools and their specific radioactivity indicated that the primary effect of methyl CCNU was on the pool size of TTP in the regenerating liver and that there was no significant inhibition of DNA synthesis by methyl CCNU.

Significance to Biomedical Research and Program of the Institute:

The mode of action of the antineoplastic nitrosoureas remains enigmatic. In the transplantable rat glioma one point of sensitivity appears at the nuclear DNA polymerase level. In the regenerating rat liver, a more easily perturbed tissue, the DNA precursor pools are increased giving only an appearance of inhibition of DNA synthesis to investigations which simply rely on radiolabelled precursor entry into DNA.

Significance to Biomedical Research and Program of the Institute (cont'd):

It is possible that the different effect of methyl CCNU may be rationalized on the basis of the metabolism of the cyclohexyl substituted nitrosoureas. The *in vivo* metabolism of CCNU to polar metabolites (Project NCI-6911) by the hepatic mixed function oxidase is rapid in normal animals such as those used in the glioma work. These tumors would be exposed primarily to polar CCNU metabolites. In contrast, it is know that an early response to partial hepatectomy is a rapid decrease in hepatic mixed function oxidase activity; the regenerating liver would thus be exposed primarily to the parent nitrosourea. The significance of these observations awaits further investigation.

Proposed Course:

To determine the relative antitumor properties of the haloethylnitrosoureas and their polar metabolites.

Honors and Awards: None.

Publications:

Hilton, J. and Walker, M.D.: Antitumor Activity of Methyl CCNU in a Transplantable Rat Glioma. The Pharmacologist 15: 234 (1973).

Presentations: None.

Serial No.: NCI-6905
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Intravenous Methyl CCNU in Malignant Central Nervous System Neoplasms (Phase II)

Previous Serial Number: None

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Marshall A. Levine, M.D.
Howard D. Weiss, M.D.
Peter H. Wiernik, M.D.

Cooperating Units: Medical Service, BCRC, NCI

Man Years:

Total: 0.3
Professional: 0.3
Other: 0

Project Description:

Objectives:

A method for delivering methyl CCNU intravenously using a 5% ethanol in a 10% fat emulsion vehicle was developed. More consistent toxicity and improved therapeutic efficacy from delivery of the parent compound were anticipated. A Phase I study determined the acceptable dose schedule to be 130 mg/m^2 in patients who had not received extensive prior radiation therapy or chemotherapy. The dose of 150 mg/m^2 was used in untreated patients. A Phase II trial of IV methyl CCNU in patients with malignant central nervous system neoplasms was undertaken.

Methods Employed:

Using 5% ethanol in 10% fat emulsion, patients previously treated with radiation therapy and/or chemotherapy received IV MeCCNU, 130 mg/m^2. Untreated patients received 150 mg/m^2. A few selected patients received 170 mg/m^2. Patients had biopsy proven malignant neoplasms unresponsive to conventional chemotherapy. Selected patients received concomitant Co^{60} cranial irradiation.

Major Findings:

The Phase II study of IV MeCCNU for malignant CNS neoplasms demonstrated therapeutic efficacy. Of 25 evaluable patients, 5 showed improvement (median = 10+ months), while 9 showed stabilization (median = 9.5 months). These responders showed a minimum progression-free interval of 6 months. These results are equivalent to the oral nitrosoureas.

IV methyl CCNU was tolerated well in doses up to 170 mg/m^2, but patients with extensive prior therapy showed a trend toward increased hematopoietic toxicity. The intravenous administration did not result in a more consistent pattern of toxicity. Ten percent of the patients had granulocyte nadirs below 1,000, while 70% had nadirs above 2,000. A similar degree of thrombocytopenia was seen. No drug toxicity deaths occurred.

Significance to Biomedical Research and Program of the Institute:

The delivery of methyl CCNU via a controlled intravenous infusion adds a new dimension to the use of highly lipid soluble nitrosoureas.

Proposed Course:

This drug is amongst several nitrosoureas demonstrating therapeutic efficacy in the treatment of malignant CNS neoplasms. It should be considered for Phase III trials. In addition, combination chemotherapy with PTG and other agents active for CNS neoplasms are being undertaken.

Honors and Awards: None.

Publications: None

Presentations:

American Society of Clinical Oncology, Houston, March 28, 1974, "Intravenous Methyl CCNU (IV MeCCNU) in the Treatment of Malignant Glioma (Phase II)", M.A. Levine, M.D. Walker, and H.D. Weiss

Serial No.: NCI-6906
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Intravenous CCNU Phase I Study

Previous Serial Number: None.

Principal Investigator: Michael D. Walker, MD.

Other Investigators: Marshall A. Levine, M.D.
Howard D. Weiss, M.D.
Philip H. Gutin, M.D.

Cooperating Units: Medical Service, BCRC, NCI.

Man Years:

Total:	0.2
Professional:	0.2
Other:	0

Project Description:

Objectives:

Oral nitrosoureas have shown irregular absorptions, toxicities, and antineoplastic affects. An intravenous preparation of methyl CCNU required a cumbersome administration technique and showed similar irregular effectiveness and toxicities. Emulfor, a non-ionic polyethylated castor oil, has been used for the administration of PTG and is a much simpler vehicle than the intralipid vehicle used for intravenous methyl CCNU (IV MeCCNU). IV MeCCNU showed comparable therapeutic efficacy to IV BCNU, oral CCNU, and oral MeCCNU. The purpose of this study was to evaluate the toxicity of intravenous CCNU using the emulfor vehicle.

Methods Employed:

Using the vehicle of 5% ethanol and 5% emulfor in D5U, starting doses of IV CCNU of 25 mg/m^2 were administered to 3 patients. After acceptable toxicity was shown, dose increments to 50 mg/m^2, 75 mg/m^2, 100 mg/m^2, and 130 mg/m^2 were administered to groups of 3 or more patients as acceptable drug toxicity was demonstrated. Patients entered had malignant neoplasms unresponsive to conventional therapy. A preponderance of malignant glioma patients were included. Selected patients had radiolabelled tracer doses for pharmacologic studies of blood, urine, CSF, and biopsied neoplastic tissue and malignant peritoneal fluid.

Major Findings:

Preclinical studies showed hematologic toxicity to be the major limiting factor. The highest non-toxic dose was 16 mg/m^2, the toxic dose low was 33-64 mg/m^2 and the toxic dose high was 250 mg/m^2. The lethal dose was 400 mg/m^2, which resulted in severe hematologic suppression.

No toxicity was seen at 25 mg/m^2 and 50 mg/m^2. At 75 mg/m^2, mild hematologic toxicity was encountered. At 100 mg/m^2, several patients with bone marrow involvement secondary to tumor showed transient thrombocytopenia to less than 20,000 and required platelet transfusions. Patients without disease involved bone marrows have generally shown WBC nadirs above 3,000 and platelet nadirs above 100,000. Three patients received 130 mg/m^2 just recently, but toxicity data is not yet available. One mixed tumor response has been noted after 25 courses of therapy. Pharmacologic studies have been performed in 2 patients. One major metabolite was seen. The ratio of the parent compound to the hydroxylated metabolite of similar structure was one-to-one after several hours. The peak plasma radioactivity was at the infusion's end.

Significance to Biomedical Research and Program of the Institute:

A simple method for delivering an aqueous insoluble compound intravenously has been developed. Toxicity has occurred at 100 mg/m^2.

Proposed Course:

The Phase I study of IV CCNU will be completed. Phase II studies are anticipated in malignant gliomas and possibly Hodgkin's disease, which has proved very responsive to oral CCNU. Further toxicity and pharmacologic studies will be undertaken.

Honors and Awards: None.

Publications: None.

Presentations: New Drug Liaison Meeting, Bethesda, December 10 and 11, 1973, "IV CCNU (Phase I)," M.A. Levine.

Serial No.: NCI-6907
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Colony Stimulating Factor (CSF) and Colony Forming Capacity (CFC) from Patients with Malignant Neoplasms, and the Effects of Chemotherapy upon CSF and CFC

Previous Serial Number: None

Principal Investigator: Marshall A. Levine, M.D.

Other Investigators: Peter H. Wiernik, M.D.
Michael D. Walker, M.D.

Cooperating Units: Medical Service, BCRC, NCI

Man Years:

Total:	0.3
Professional:	0.3
Other:	0

Project Description:

Objectives:

Since the 1960's, numerous reports of the successful in vitro growth of human peripheral blood and bone marrow have been published. In small groups of leukemia patients, CSF and CFC have been shown to rise during remission and fall during relapse. In addition, leukemic cells have been shown to mature in vitro on occasion. The large leukemic population of the Baltimore Cancer Research Center would allow more adequate assessment of these findings. Additionally, the mechanism of delayed cumulative hematologic suppression with nitrosourea therapy has not been explained. The in vitro culture technique would allow us to determine if increased CSF (stimulatory) or decreased CFC (target) is the basic deficit caused by nitrosourea chemotherapy. The large malignant central nervous system neoplasm patient population of the BCRC would allow a unique opportunity to study this peculiarity of the nitrosoureas. Primary central nervous system neoplasm patients rarely have metastases to bone marrow producing areas. The following assessment will be made:

1. Measurement of CSF and CFC in various stages of hematologic malignancy.

2. Measurement of CSF and CFC in various stages of solid tumors

Objectives (cont'd)

infiltrating bone marrow.

3. Measurement of CSF and CFC in patients in various stages of chemotherapy, particularly with nitrosourea compounds.

4. Morphologic studies of *in vitro* grown leukemic cells.

Methods Employed:

The growth technique involves incubation for a short period of time a source of CSF such as urine, peripheral white blood cells, plasma or serum in McCoy's media, fetal calf serum, and dilute agar or methyl cellulose. Bone marrow from human beings or mice are evenly dispersed in the same semisolid culture medium and incubated over the prepared feeder layers. The cultures are incubated in 35 mm petri dishes for 10-21 days in a humidified CO_2 incubator at 37°C, removed and examined under a dissecting microscope. Culture dishes are examined for colony counts and cell morphology.

Major Findings:

The project is in its early stages. Using agar as a solidifing source, inconsistent cell growth has been achieved. Better consistency is needed. Additionally, serum, plasma, and urine samples from patients in various stages of disease have been accumulated.

Significance to Biomedical Research and Program of the Institute:

In vitro studies of the various factors involved in hematologic cell growth would be of scientific significance.

Proposed Course:

Further refinements in the culture techniques are anticipated. When consistent growth is obtained, detailed studies of hematopoietic function will be undertaken. We also hope to develop some information regarding tumor burden levels in the bone marrow.

Honors and Awards: None.

Publications: None.

Presentations: None.

Serial No.: NCI-6908
1. Baltimore Cancer Research Center
2. Section of Neurosurgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Intravenous Methyl-CCNU (NSC-95441) and PTG (NSC 122819) in the Treatment of Central Nervous Neoplasms (Phase II)

Previous Serial Number: None

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Philip H. Gutin, M.D.
Howard D. Weiss, MD.

Cooperating Units: Medical Service, BCRC, NCI

Man Years:

Total:	0.3
Professional:	0.3
Other:	0

Project Description:

Objectives:

The encouraging results and limited toxicity associated with the individual use of these agents leads us to determine the therapeutic efficacy of intravenous methyl CCNU and epipodophyllotoxin (PTG) used in combination in the treatment of central nervous system neoplasms.

Methods Employed:

Patients with biopsy-proven or clear-cut clinical evidence of intracerebral malignancy who have not received prior cytotoxic chemotherapy may be included in this study. All patients must have evidence of an adequate bone marrow reserve. Patients may receive cranial irradiation. Each course of therapy begins with a dose of IV MeCCNU (130 mg/m^2) and PTG (100 mg/m^2) on day 1, followed by 5 additional doses of PTG (100 mg/m^2) on days 8, 15, 22, 29 and 36. Subsequent courses begin 2 weeks after the last PTG dose and the cycle then repeats itself. The dose of IV MeCCNU is reduced to 100 mg/m^2 if the WBC nadir is <1500 or the platelet nadir <30,000 on the preceeding course. Therapy continues until disease progression or unacceptable toxicity occurs.

Major Findings:

To date 12 patients with primary malignant brain tumors have been entered on this study. Seven patients have continued to deteriorate despite therapy, 4 of whom have died and 3 are on other BCRC protocols. One patient has remained in a state of "continued well being" for the 6 months since therapy was started. Four patients are considered not evaluable due to inadequate length of follow up.

Significance to Biomedical Research and Program of the Institute:

This is one of the first attempts to use in combination two drugs which have been shown individually to be effective against cerebral neoplasms.

Proposed Course:

Completion of Phase II study in next few months.

Honors and Awards: None.

Publications: None.

Presentations: None.

Serial No.: NCI-6909
1. Baltimore Cancer Research Center
2. Sections of Neurological Surgery and Medicine
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Phase I Evaluation of Intrathecal N,N',N"-Triethylenethiophosphoramide (Thiotepa-NSC-6396) in Meningeal Neoplasms

Previous Serial Number: None

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Howard D. Weiss, M.D.
Peter H. Wiernik, M.D.
Philip H. Gutin, M.D.

Cooperating Units: Medical Service, BCRC, NCI

Man Years:

Total:	0.3
Professional:	0.3
Others:	0

Project Description:

Objectives:

To determine the maximally tolerated dose of thiotepa intrathecally, the parameters of its toxicity, and an indication of its therapeutic efficacy in meningeal leukemia, lymphoma, and carcinoma.

Methods Employed:

Patients with meningeal infiltration by leukemia, lymphoma, or carcinoma who are considered no longer appropriate for conventional therapy receive twice-weekly doses of intrathecal thiotepa. The dose in initial patients was 1 mg/m^2 (1/6 the "toxic dose low" in monkeys) with later escalations to 2, 4, 6, 8, and 10 mg/m^2 after sufficient evaluation at the lower dose indicated its safety. After 2 doses the dose could be escalated one step in an individual patient (only 1 dose escalation allowed per patient). Treatment is continued until response, failure, or bone marrow toxicity occurs.

Major Findings

Eleven patients received a total of 70 treatments with intrathecal thiotepa at doses ranging from 1-10 mg/m^2/twice weekly. No serious or permanent

Major Findings (cont'd)

neurotoxicity observed, although 1 patient treated at 4 mg/m^2 and 2 treated at 10 mg/m^2 had transient paresthesias during and briefly after the injections. No one developed significant or unusually prolonged myelosuppression which could be attributed to thiotepa alone, but all patients were receiving concurrent systemic chemotherapy so this was difficult to accurately evaluate.4 patients (all adults with ALL) had complete remission of meningeal leukemia following 3-6 injections at doses from 2-10 mg/m^2; 3 patients (1 PDL, 2 ALL) had partial but clinically insignificant responses; 1 patient (adult ALL) clearly progressed during therapy; the clinical responses of 3 patients (1 ALL, 1 ependymoma, 1 lymphoma) were not evaluable.

Significance to Biomedical Research and Program of the Institute:

Intrathecal thiotepa has little toxicity and some evidence of therapeutic activity and is a promising new agent for the treatment of meningeal neoplasms.

Proposed Course:

Phase II studies of intrathecal thiotepa in patients with meningeal neoplasms.

Honors and Awards: None.

Publications: None.

Presentations:

American Association for Cancer Research, Houston, March 28, 1974, "Preclinical and Phase-I Clinical Studies of Intrathecal N,N'N"-Triethylenephosphoramide (Thiotepa NSC-6396)", H. D. Weiss, M.D. Walker, P.H. Wiernik, and D. Dalgard.

Serial No.: NCI-6910
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: *In Vitro* Metabolism of CCNU and Methyl CCNU

Previous Serial Number: NONE

Principal Investigator: John Hilton, D.Phil

Other Investigators: Michael D. Walker, M.D.
Donald L. Bowie, B.S.
Deborah Zito, Student

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.3

Project Description:

Objectives:

To determine the nature and rate of formation of CCNU and Methyl CCNU metabolites produced during the action of mixed function oxidase *in vitro*.

Methods Employed:

The products of the action of a mixed function oxidase preparation on CCNU and Methyl CCNU are extracted into an organic solvent. The extract is analyzed by thin layer and high pressure liquid chromatography. On a preparative scale sufficient amounts of metabolites to allow chemical characterization can be generated.

Major Findings:

CCNU and Methyl CCNU are rapidly converted by a mixed function oxidase preparation to a number of polar metabolites which retain the basic structure of the parent compound.

Significance to Biomedical Research and the Program of the Institute

The conversion of CCNU and Methyl CCNU to more polar metabolites still re-

Significance to Biomedical Research (cont'd)

taining the basic structure of their parent compound is a new observation which will modify most preconceptions on the pharmacology of these antineoplastic agents.

Proposed Course:

To characterize the early *in vitro* metabolites of CCNU and Methyl CCNU and assign empirical and stereochemical structures.

Honors and Awards: None

Publications: None

Presentations: None

Serial No.: NCI-6911
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21211

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: In vivo metabolism of 3-Cyclohexyl Substituted in 1-Chloroethyl 1-Nitrosoureas

Previous Serial Number: None

Principal Investigator: John Hilton, D. Phil.

Other Investigators: Michael D. Walker, M.D.
Donald L. Bowie, B.S.
Deborah Zito, Student

Cooperating Units: None

Man Years:

Total:	0.7
Professional:	0.4
Others:	0.3

Project Description:

Objectives:

To determine the nature, rate of formation and disposition of polar metabolites of 3-cyclohexyl substituted 1-chloroethyl 1-nitrosoureas.

Methods Employed:

Plasma samples from rats receiving IV CCNU or methyl CCNU are subjected to solvent extraction. The extracts are analysed by thin layers and high pressure liquid chromatography.

Major Findings:

There occurs a very rapid conversion of the nitrosoureas to polar metabolites which retain the basic structure of the parent molecule. The rate of metabolite formation is enhanced in animals pretreated with phenobarbital. The metabolites found in vivo appear identical to those found in vitro (Project NCI-6910).

Significance to Biomedical Research and Program of the Institute:

The appearance in plasma of polar metabolites of CCNU and methyl CCNU having

Significance to Biomedical Research and Program of the Institute (cont'd)

the same basic structure of the parent compound has not been reported in the literature. The detection and characterization of these metabolites will be an important step toward rationalization of the pharmacokinetics and antineoplastic properties of the parent compounds.

Proposed Course:

To characterize chemically the polar metabolites of CCNU and methyl CCNU, to determine their rate of formation in various tissues and to investigate their disposition in normal and neoplastic tissues.

Honors and Awards: None.

Publications: None.

Presentations: None.

Serial No: NCI-6043
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Automated Monitoring of Drug Therapy

Previous Serial Number: Same

Principal Investigators: Clarence L. Fortner, James K. Hooper

Other Investigators: Michael D. Walker, Richard Hsieh, William R. Grove, Peter H, Wiernik

Cooperating Units: Division of Health Services Research, Baltimore USPHS Hospital; Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 2-1/2
Professional: 1/2
Other: 2

Project Description:

Objectives:

To provide inexpensive, efficient and rapid methods of collecting data concerning the clinical trials of experimental drugs.

Methods Employed:

An automated method of ordering and recording drug therapy was designed and implemented.

An inexpensive system of processing drug information provides accurate, weekly summaries of each patient's drug therapy. The physician writes each drug order directly on a medication card, producing four copies: the medicine card copy, the pharmacy copy (for entry to a patient profile), and copies for medical records and automated analysis. The nurse charts medications given directly on the first copy of the drug form at the time of drug administration. A clerk types the drug name, dosage information, patient name and record number and date drug was initiated. This data is read weekly by optical character recognition. The data is collated and patient drug summaries are printed listing drug, dose, total dose, frequency of administration and a daily record of administration of omission of each drug. A drug utilization report is also obtained as a by-product.

Methods Employed (cont'd)

The problem number and statement according to the Weed's problem oriented health record system has been incorporated into the form which allows each drug to be listed by problem number on the weekly drug summary for each patient. Space on the drug form has also been allotted for the patients' height, weight, and body surface area. This gives the pharmacist enough data to recalculate all drug doses listed in research protocols. Not only does this recalculation protect the patient, but it also protects the research environment by insuring the accuracy of all doses administered.

Major Findings:

The medication card has proven to fulfill all the requirements of drug ordering, administration and recording, in addition to providing machine-readable data for computer input. The summaries are checked by physicians and pharmacists and become a permanent record of drug therapy both in the patient's chart and in the pharmacy. This system eliminates the transcription operations usually associated with preparation of input for computers, and provides a data pool for clinical studies, analyzing drug effect, toxicity, and drug interaction. This system has reduced errors in drug administration and has increased staff awareness of the problems inherent in quality drug administration. A complete list of all drugs administered to the patient are recorded by problem numbers in a concise and orderly fashion in the patient chart.

Significance to Biomedical Research:

This system, as outlined, has provided a more comprehensive and efficient record of cancer chemotherapy drug trials.

Honors and Awards: None

Publications: None

Serial No: NCI-6236
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Role of the Pharmacist in Obtaining a Patient's Drug and Allergy History

Previous Serial Number: Same

Principal Investigator: Clarence L. Fortner

Other Investigators: William R. Grove and Robert De Christoforo

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 1/8
Professional: 1/8
Other: 0

Project Description:

Objectives:

To evaluate the feasibility of having a pharmacist interview all patients admitted to the Baltimore Cancer Research Center for the purpose of obtaining a complete drug and allergy history.

Methods Employed:

The pharmacist questions each patient admitted to the Medicine and Neurosurgical Services, BCRC, concerning current and past medication usage and history of allergy.

A written record of this interview is placed in the history section of the patient's chart and in the pharmacy's drug profile for each patient.

Major Findings:

Pharmacists recorded drug and allergy histories have resulted in more complete drug and allergy history information. Some of the results obtained from these interviews are summarized: patients taking prescription drugs at the time of admission-75%, patients taking prescription drugs during the past year-90%, patients taking non-prescription drugs within a few weeks of admission-92%, and patients reporting a history of allergy to drugs, food,

Major Findings (cont'd)

animals, clothing, tape, and iodine-318. The interview includes determining all of the over the counter (OTC) drugs that the patient has received without professional supervision. This information is extremely valuable at the time the patient is discharged from the hospital. The pharmacist is able to utilize this information by advising the patient of any drug-drug interactions, etc. between the OTC drug and drugs being prescribed by the physician upon discharge. These interviews have provided the pharmacist with patient contact at the time of admission which has increased the pharmacist awareness of patient care. All medications brought to the hospital by the patient are obtained at time of the interview, and are stored in the pharmacy during the hospitalized period. The pharmacists are responsible for placing all known allergic reaction information on the front of the patients drug Kardex, and on the front of the patient chart. At the time of completion of the interview, the original is placed in the history section of the patients hospital chart and a duplicate is attached to the patients drug profile in the pharmacy. If the pharmacists feel that some of the information should be brought to the physicians attention immediately, he also communicates verbally to the physicians. These interviews have demonstrated that the pharmacist is interested in patient care, and that he can provide an additional useful service. The drug and allergy interview by the pharmacist has become an integral part of the clinical pharmacy program.

Significance to Biomedical Research:

More complete histories of medication usage are provided which may result in improved care of patients.

Proposed Course:

The present study will be continued.

Honors and Awards: None

Publications: None

Serial No: NCI-6237
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Development of a Patient Care Pharmacy Service

Previous Serial Number: Same

Principal Investigator: Clarence L. Fortner

Other Investigators: William R. Grove and Robert De Christoforo

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 1-1/3
Professional: 1-1/3
Other: 0

Project Description:

Objective:

The pharmacist has traditionally been a product oriented person with little input to the actual care of the patient. The objective of this study is to determine the role of the pharmacist in the patient care area, and as a member of the health care team actively contributing to the patient's care. Patient education has been added to this study. The objective of this education will be to increase compliance and reduce problems for outpatients receiving drugs.

Methods Employed:

The pharmacist is located in the midst of the patient care area in order to allow him to investigate new concepts, approaches, and research opportunities to aid in the patient care. The pharmacist attends morning and evening ward rounds, reviews charts, interviews patients and is familiar with the patients' condition. Therefore he can intelligently discuss drug and therapy problems with the patient's physician. With this information, he can also provide the staff with drug information.

An integral part of this service is the drug surveillance system. The nucleus of the drug surveillance system is the individual patient drug profile. The pharmacist receives a direct copy of the physician drug order and transcribes it to the patient drug profile. The drug profile includes the patient name, primary and secondary problems, physician name, known allergies or idiosyn-

Methods Employed (con't)

crasies and all drug orders. The pharmacist reviews each new drug order for completeness and recalculates all protocol drug dosages then reviews the drug profile against the new order for drug interaction, laboratory problem, dietary problem and possible adverse drug reactions. The drugs are then dispensed thru a unit dose system which provides accurate records. All returned doses to the pharmacy are investigated. The monitoring system involves evaluating the necessity of continued medication orders.

An extensive library, including an drug abstracting service, aids the pharmacist in performing an additional service to the BCRC, one of drug information. Physicians questions are research and answered promptly, to aid in their ability to evaluate patient care.

Major Findings:

The pharmacist has proven that he can be patient oriented rather than product oriented. The pharmacist utilizing the above methods has become a valuable member of the health care team and contributes directly to the patient care. The following has been accomplished: improving drug prescribing practices by promoting the safe and rational use of drugs, preventing potential drug-drug interactions, detecting and preventing adverse drug reactions, detecting drug-induced laboratory test abnormalities, detecting possible drug-induced diseases, providing drug information, counseling the patient on proper use of drugs, and detecting and preventing intravenous admixture incompatibilities. This has reduced drug exposure to the patient, decreased nursing and pharmacy time spent administering and preparing drugs and reduced cost.

Significance to Biomedical Research:

A higher quality of drug prescribing and reduced medication errors has contributed toward better patient care and has enhanced the quality of the research data.

Proposed Course:

Present studies will be continued and additional methods to improve patient care, such as unit dose drug distribution, will be further developed.

Honors and Awards:

American Pharmaceutical Association Military Section Literary Award, 1973 (The Acute Leukemias: Journal of the American Pharmaceutical Association: NS12;9;473-481; (Sept) 1972)

Publications:

De Christoforo, R. and Fortner, C.L.: A Review of Potential Drug Interactions Between OTC and Prescription Drugs; Hospital Formulary Management, April 1974

Serial No: NCI-6238
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Development of a Unit Dose Service and the Preparation of Parenteral Drugs by a Patient Care Pharmacy Service

Previous Serial Number: Same and 6269

Principal Investigator: Clarence L. Fortner

Other Investigators: William R. Grove and Robert De Christoforo

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 3-1/2
Professional: 2
Other: 1-1/2

Project Description:

Objectives:

To improve patient care through a new drug delivery system and to provide more accurate information regarding the disposition of drugs.

Methods Employed:

A unit dose of medicine is a single dose of medicine packaged for an individual patient. This single dose package is labelled with a name, strength, and control number of the drug, and it is ready to be administered to the patient. These drugs are ordered by the physician and they are placed in the individual patients drawer of a medication cart by the pharmacist. This cart is designed so that there is a single drawer for each patient's drug. All of the parenteral products are prepared aseptically in a laminar air flow environment, and are dispensed in a clearly labelled ready to use form. Parenteral products are prepared for not more than a 24 hour period to ensure full potentcy. The parenteral drugs are not placed in this cart. They are carried directly to a refrigerator in the nursing unit. Control procedures for checking the composition of the product prepared, and for monitoring bacterial and particulate matter contamination of these preparations have been instituted. Pertinent information regarding the drugs dispensed is recorded on specially designed forms that are filed according to the patients name. This information is also recorded by drug and lot number for each investigational agent.

Major Findings:

The two systems, unit-dose and I.V. admixture, have proven to be advantageous over the old ward stock system of drug distribution. They have placed the preparation and distribution in the hands of the pharmacist. These systems have accomplished the following: reduced medication errors, reduced medication cost, enhanced the pharmacist as a drug information consultant by relating him more closely to the patient, reduced the possibility of contamination of the drug, permits nurses to use time more effectively in direct patient care and in general allows the hospital to demonstrate adequate control over the distribution of all drugs. In addition, the pharmacist has instituted record systems that have improved retrieval of the patient's drug record. Physicians utilize these records daily in ordering medications for patients and have found them to be a useful and accurate source of information.

During the past year the pharmacy service has dispensed approximately 283,842 oral doses of medication to inpatients, 24,413 I.V. admixtures, and 6,887 doses of parenteral investigational cancer chemotherapeutic agents.

Significance to Biomedical Research:

By reducing medication errors, and improving the accuracy of the records of disposition of cancer chemotherapeutic agents and the other unit dose drugs the quality of patients care has increased. These delivery systems have been intergreted with the development of the Patient Care Pharmacy service which is providing the patients with better care. Thus, the research drug data will be improved.

Honors and Awards: None

Publications:

Fortner, C.L., and Grove, W.R.: A Unit-Dose System Intergrated With a Clinical Pharmacy Service in a Cancer Research Center; Hospital Formulary Management, Vol. 8, No. 6, 43-49, June 1973

Fortner, C.L., Bowie, D., and Walker, M.D.: Intralipid^R As A Vehicle for I.V. Administration of an Aqueous Insoluble Drug; Clinical Society and Commissioned Officers Assoc. of USPHS, 19A, (Apr) 1974

Serial No: NCI-6912
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Antineoplastic Drug Displays for the Computerized Problem-Oriented Medical Information System (PROMIS)

Previous Serial Number: None

Principal Investigator: William R. Grove

Other Investigators: Paul Chang, Robert Esterhay, Clarence Fortner, George E. Nelson, Peter H. Wiernik

Cooperating Units: Medicine and Neurosurgical Service, BCRC; PROMIS Laboratory, Dept. Medicine, University of Vermont

Man Years:

Total: 1/8

Professional: 1/8

Other: 0

Project Description:

Objectives:

To develop accurate, complete and concise antineoplastic drug displays for addition to the computerized drug displays already on file with the PROMIS system, Burlington, Vermont. These displays are then retrievable at the touch-sensitive cathode ray screen terminal located at the nursing station at the BCRC.

Methods Employed:

Literature research is conducted to obtain accurate and complete drug information on antineoplastic agents. This information is then consolidated to a specific format to conform with the over 20,000 displays currently on the PROMIS system. The format consists of separate display pages for information as follows: DRUG NAME, CHECK PROBLEM LIST FOR, SIDE EFFECTS TO WATCH FOR, DRUG INTERACTIONS, TEST INTERFERENCES, USUAL DOSAGE, PHARMACEUTICAL DATA, MECHANISM OF ACTION, METABOLISM AND EXCRETION. All statements are fully documented to the specific reference source. Each completed display sequence is then sent to the PROMIS laboratory for auditing for accuracy, completeness, and correctness of clinical judgement before being entered into the computerized system.

Serial No: NCI-6912

Major Findings:

New drug displays have been written and entered into the system.

Significance to Biomedical Research:

The antineoplastic drug displays summarize the current medical literature into concise, accurate, and complete displays of pharmacologic information of antineoplastic agents. The completed drug displays form an integral part of the contribution to improved patient care and improved medical records played by the PROMIS system.

Proposed Course:

Continued study, with additional antineoplastic drug displays being completed and entered into the computerized PROMIS system.

Honors and Awards: None

Publications: None

Serial No. NCI 6023
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30,1973

Project Title: Radiation Therapy Service

Principle Investigators: Roger W. Byhardt,M.D., and Kirkland C. Brace,M.D.

Other Investigators: Joan M. Zarriello, R.N. and Patricia K. Alemi

Cooperating Units: Baltimore Cancer Research Center and the U.S. Public Health Service Hospital, Baltimore, Maryland

Man Years: (July 1, 1972 - June 30, 1973)

Total: 3
Professional: 1
Others: 2

Project Description:

Objectives:

The Radiation Therapy Service is an integral part of the Baltimore Cancer Research Center treatment program. The protocols during the past year have been mainly concerned with glioblastoma multiforme and acute leukemia. The patients in these two studies receive radiation therapy as a part of a combined treatment program. Patients with other types of tumors are treated to control various aspects of their disease. The general hospital continues to have an active Tumor Service which utilizes both pre and post-operative radiation as part of the routine treatment of cancer patients.

Methods Employed:

The equipment presently consists of a 100 kv. superficial therapy machine, a 285 kv. orthovoltage machine and a Theratron-80 cobalt unit. The 6 million volt linear accelerator will not be installed at this institution but will be installed at the University of Maryland Hospital.

Major Findings:

The number of patients treated during the past year has increased significantly over past years mainly because of the increased activity of the research program. During the year ending March 31, 1974 a total of 264 patients were seen in consultation and 234 were accepted for treatment. Of this total treated 192 patients came from the research service.

The breakdown of the treatments follow (April 1, 1972 - March 31, 1973):

	Tumor Service	Hospital Service
Superficial Therapy Treatments	18	59
Orthovoltage Therapy Treatments	39	18
Cesium^{137} Applications	0	4
Strontium^{90} Applications	0	3
P^{32} Injections	3	0
Cobalt^{60} Treatments	2976	1395

Significance:

The progressive decline in the general hospital population continues to reduce the number of Service patients treated at this facility. This loss has not reduced the activity significantly because of the increase in the number of cancer research patients treated during the past year.

Course:

The Radiation Therapy Service at this institution will cease to exist as of July 1, 1974 for all patients. The research facilities will be transferred to the University of Maryland Hospital. It is hoped that this will permit an increase in the number of patients and the enlarged facilities should provide opportunity for better treatment.

Honors & Awards:

None

Publications:

None

Serial No. NCI 6207
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1,1972 through June 30,1973

Project Title: Radiological Physics - A Special Service Which is Supplied for Research Purposes

Principal Investigator: Roger W. Byhardt,M.D.

Other Investigators: Joan M. Zarriello, R.N. and Patricia K. Alemi

Man Years: (July 1, 1972 through June 30, 1973)

Total: 1/8
Professional: 1/16
Other: 1/16

Project Description:

Objectives:

To provide dosimetry for the treatment of patients.

To provide non-patient service irradiation for the laboratory.

To provide a Radiation Safety Program for the Pharmacology Laboratories and the Radiotherapy Department.

Methods Employed:

The radiation therapy machines are calibrated periodically to insure accuracy in the amount of radiation administered. Treatment plans are devised and checked with thermoluminescent dosimeters as needed for the patients.

Radiation is provided for both cellular and small animal work from the Pharmacology Laboratories and for the Johns Hopkins Laboratory of Biology. A total of 100 exposures were provided of high energy irradiation. A total of 21 exposures to low energy x-rays were provided during the past year.

The Radiation Safety Program in the Radiation Therapy Department and the Pharmacology Laboratory is maintained at a minimum level. The sealed sources are routinely examined for leakage as required by A.E.C. Regulations and laboratories are surveyed with wipe tests to insure cleanliness of all facilities.

Major Findings:

There have been no detectable leakage of any of the sealed sources during the year. There have been several minor spills in the laboratory, none of which were of any significant level.

Proposed Course:

The radiation facilities of the Baltimore Cancer Research Center will be closed on or about July 1, 1974. We expect the general services to be carried on by the Physics Department at the University of Maryland Hospital.

Honors & Awards:

None

Publications:

None

Serial No: NCI 6270
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Combination Chemotherapy and Radiotherapy in the Treatment of Hodgkin's Disease

Principal Investigator: Roger W. Byhardt,M.D., Acting Head, Radiotherapy

Other Investigators: Kirkland C. Brace,M.D., Consultant in Radiotherapy
Joan M. Zarriello, R.N., Nurse-Technician in Radiotherapy

Cooperating Unit: Medicine Service, Baltimore Cancer Research Center

Man Years: (July 1, 1972 - June 30, 1973)

Total: 2
Professional: 1
Others: 1

Project Description:

Objectives:

The initial study of Stage I and II Hodgkin's disease clearly reveals that the radiation to involved areas followed by combination chemotherapy MOPP was clearly superior to the radiation alone and the chemotherapy followed by radiation. A second protocol using only radiation of the involved plus adjacent lymph nodes compared to irradiation of the involved lymph nodes plus chemotherapy is now almost complete. This randomized study now totals 84 patients who ar who are available for at least two years follow-up. We hope that this will provide a significant improvement in the survival of all Hodgkin's disease patients.

Methods Employed:

Stage I, II, and III-A Hodgkin's disease patients were randomized into two groups. Each patient underwent exploratory laporatomy for thorough staging of the disease and each patient had a splenectomy. One half of the patients received irradiation to the known tumor-bearing areas followed by three to six courses of chemotherapy and the other half received irradiation to the involved lymph nodes plus adjacent lymph node-bearing areas.

Major Findings:

More than 80 patients have been randomized in this study during the past three years. It is now clear that the chemotherapy is capable of controlling disease outside the areas irradiated but the survival data does not, as yeat, reveal whether or not one group is better than the other.

Significance:

The combination of MOPP and radiation may prove to be an important advance in the treatment of Hodgkin's in the early as well at the late stages.

Course:

The sutdy will be continued on a modified protocol which will permit patients to be treated without the exploratory laporatomy. In this way it may be possible to show that this operative procedure is unnecessary for the adequate treatment of this disease.

Honors & Awards:

None

Publications:

Brace, K., Serpick, A.A., Block, J.B., and Wiernik, P.H.: Combination Radiotherapy and Chemotherapy in the Treatment of Early Hodgkin's Disease. Oncology 27: 484-492 (1973).

SUMMARY REPORT

LABORATORY OF PHARMACOLOGY

Efforts in the Biochemistry Section have been directed in a broad program of basic and clinical research of cancer chemotherapeutic agents as well as in the study of the controlling factors of cell growth and differentiation. In the studies on the metabolism of anthracycline antibiotics, daunorubicin and adriamycin reductase have been purified from rat and rabbit tissues using affinity column chromatography. Studies on the properties of the purified enzymes are ongoing.

That the metabolism of daunorubicin and adriamycin is as yet not fully understood comes from the isolation of several new and not yet fully characterized compounds from the urine of patients receiving these antibiotic agents. Of particular interest are the demethylated adriamycin and daunorubicin aglycones as well as the demethyl 4-O-sulfate and glucuronide conjugates of these aglycones.

An additional point of interest with respect to the metabolism of these substances is that cytofluorescence techniques have revealed the intercellular localization of both adriamycin and daunorubicin to be primarily in the nucleus.

Collaborative studies with the Medical Service on the clinical pharmacology of the tetracycline antibiotics have also been continued.

Since it has been previously shown that adriamycin has a long plasma half-life and is excreted primarily in the liver, comparison of the clinical and pharmacologic data from 96 patients treated with adriamycin has shown that patients with significantly impaired liver function had prolonged and severe toxicity which correlated well with prolonged plasma and drug levels. It has been concluded that adriamycin can be used with relative safety and high efficacy in the dosage schedule that was obtained from the results of our pharmacologic studies. Dosage reduction in patients with liver disease is essential to avoid life-threatening toxicity.

In studies on the cellular control mechanisms affecting cell growth and differentiation, concentration has been on the importance of techniques for the isolation of liver parenchymal cells. It has been found that by measuring endogenous respiration of isolated liver cells it is possible to obtain a better estimate of the metabolic status of these cells. When isolating the cells it was also shown that a high oxygen level and strict control of the pH of the incubation medium was necessary to obtain cells with metabolic activity similar to the liver cells _in vivo_.

A method has been developed for the chemical conversion of plasma prostaglandins PGA and PGE to PGB and with commercial antisera have measured PGB equivalents in human plasma by the radioimmunoassay technique. Although patients with tumors of the testes, prostate, larynx, pharynx, and stomach showed a significant decrease of PGB levels, only one patient with pancreatic carcinoma had a tenfold increase in PCB levels. Patients with nontumor conditions, including hypertension, showed no significant differences from the normal.

Within the Enzymology Section, concentration has been on gaining a greater understanding of the physiological role of polyamines in regulating cell metabolism. Previous work from the Section and elsewhere has shown a strong correlation between the presence of polyamines and RNA synthesis. Since the concentration of the RNA within the cell is not only a function of the amount synthesized, but also of the amount degraded, examination of the relationship between these substances and degradative enzymes (the ribonucleases) was undertaken. Thus far three ribonucleases and one deoxyribonuclease have been studied. In all four, enzyme activity has been increased from three- to tenfold in the presence of the polyamines, particularly spermidine. In one, isolated from Citrobacter sp., the specificity of the enzyme has been altered in that the hydrolytic attack, previously confined to uridylic acid residues, is now broader in scope, attacking other residues as well. In two others (a deoxyribonuclease and a human plasma ribonuclease) spermidine appears to be necessary to maintain the integrity of the protein. Thus the molecular weights of these proteins are altered significantly in the presence of the polyamine. Finally, the polyamines bind to all four proteins and, in fact, [^{3}H] spermidine bound peptides have been isolated from each enzyme. Amino acid analysis of the peptide has revealed in every case the existence of a nucleophilic center to which the positively charged cations are attached. These amino acids are serine, aspartic and glutamic acids.

In view of the earlier findings that polyamines in human urine may have diagnostic importance in detecting neoplasia in man, the isolation of a protein (human plasma ribonuclease) from human plasma that can bind the polyamines is of considerable significance. The enzyme appears to be in particularly high concentration in multiple myeloma although the clinical implications of this are as yet unclear.

Ongoing studies within the Section on specific ribonucleases and deoxyribonucleases have been of importance in the elucidation of the termini of an RNA isolated from AKR murine leukemia virus. Thus uridylic acid has been found at the 3' terminus, whereas adenylic acid is at the 5' terminus of the long chain polymer. Currently studies are being undertaken to examine the polarity of the viral RNA, particularly with respect to the section of the polymer coding for reverse transcriptase.

In collaboration with the Oncology Service of the Baltimore City Hospitals, studies on the use of polyamine levels in human urine as an indicator of the presence of neoplasia are ongoing. Thus far from the results obtained with patients undergoing extensive chemotherapy, what appears to be of particular significance in evaluating the disease's state is the concentration of spermidine.

Immunologic research has concentrated on in vitro and in vivo systems designed to detect both host response to tumor-specific antigens as well as the amplification of responses to tumor-specific antigens. Previous observations have indicated that the double-stranded synthetic polyribonucleotide, polyadenylic-polyuridylic acid, was capable of amplifying lymphocyte immune responsiveness *in vitro*. Advantage has been taken of this observation in a study in which the effect of the chronic administration of polyadenylic-polyuridylic acid was carried out in the AKR mouse as both a preventive and therapeutic measure to inhibit or delay the onset of spontaneous leukemia in this strain. Poly AU shifted the median survival of female AKR mice in this model by over 4 1/2 months. The lack of toxicity of this agent plus evidence suggesting that more frequent administration at higher doses may result in more effective immune amplification suggests that poly AU may elicit even greater longevity in the AKR model. This agent is important because it represents a class of biologic compounds whose mechanism of effectiveness may be the key to developing more improved mechanisms for the control of the immune system. Research has also been undertaken defining the role of complement in mediating tumor cell lysis *in vivo* and *in vitro*. Of importance has been the observation that a close homology must exist between tumor-specific antibody and complement in order for tumor destruction to take place. Of equal importance, however, has been the observation that complement can mediate the cross-reactive binding of tumor-specific antiserum to normal cellular antigens. This phenomenon cannot take place if antibody is absorbed with these determinants in the absence of an active homologous complement source. Complement activity in tumor-bearing mice has been evaluated. These studies indicate that complement progressively decreased as the tumor burden increased. The prerequisite that complement have homology with the antibody source in mediating cell kill as well as the findings indicating that tumor-bearing animals become deficient in complement activity are important in further characterizing those aspects of antibody use which may be effective in mediating cell kill *in vivo*. Work has also been undertaken to determine host response *in vitro* to viral particles using the lymphocyte blastogenesis system. The model utilized has been rubella. Comparison of lymphocyte responsiveness to rubella and the presence of hemagglutination inhibition antibody to the same agent was made. Data was obtained which indicated that lymphocyte responsiveness (based on lymphocyte memory) was a more sensitive system to detect previous exposure to virus. At present attempts are being made to implement such assays in the study of previous exposure to putative oncogenic virus in patients with neoplasia.

Serial No. NCI-6012
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1974

Project Title: Biochemical Pharmacology of Cancer Chemotherapeutic Agents

Previous Serial Number: NCI-6012

Principal Investigator: Nicholas R. Bachur

Other Investigators: Ronald Felsted
Shigeru Takanashi
Marta Kaber
Malcolm Gee
Emanuela Arena

Cooperating Units: None

Man Years (July 1, 1973 to June 30, 1974)

Total: 5.0
Professional: 3.0
Other: 2.0

Project Description:

Objectives:

To study the biochemical pharmacology of selected cancer chemotherapeutic agents and to provide a scientific foundation for their usage. To resolve the metabolic pathways of cancer chemotherapeutic agents and to study the interactions of these drugs and their metabolites with tissue components *in vitro* and with organized physiological systems *in vivo*.

Major Findings:

Daunorubicin reductase after being purified and isolated, has been studied in an effort to understand the normal *in vivo* function of this apparently ubiquitous mammalian enzyme. The enzyme was purified from rat liver by ammonium sulfate fractionation, DEAE cellulose, hydroxyapatite, and BioGel P200 column chromatography with an overall recovery of 46%. On the basis of polyacrylamide electrophoresis with and without sodium dodecyl sulfate, gel filtration chromatography, and high speed ultracentrifugation, the final preparation is judged homogenous. Sedimentation equilibrium analysis and sodium dodecyl sulfate polyacrylamide electrophoresis give molecular weights of 38,796 ± 369, and 39,800 respectively, and suggest a single polypeptide chain. In agreement is a molecular weight of 39,541 calculated from the proposed amino acid composition. The enzyme had an isoelectric point of 6.3 and a strict requirement for NADPH. The daunorubicin-NADPH interaction occurs with a 1:1 stoichiometry. The apparent equilibrium constant at pH 8.6 for reduction is $2.9 \times 10^9\ M^{-1}$, and the apparent K_m for daunorubicin is 57 μM. Several common sulfhydryl reactants inhibited the enzyme. A closely related antibiotic, adriamycin, is reduced at about 5% the rate of daunorubicin reduction. In addition to the antibiotic, the enzyme reduces some aldo sugars, particularly, D-glucuronolactone, D-glyceraldehyde, and the isomers, D-glucuronate and D-galacturonate. D-glucuronolatone and daunorubicin were the preferred substrates.

We have succeeded in purifying and isolating several new metabolites of daunorubicin and adriamycin for human urine. By the use of polymeric sorbants, a specific method for the extraction of the anthracycline metabolites from urine has been developed. In addition, a new technique of droplet counter-current chromatography has been applied to the purification of these metabolites.

Significance to Biomedical Research and Program of the Institute:

The understanding of the biochemical alterations that active chemotherapeutic agents undergo is central to the understanding of their usage. This is of importance with the anthracycline antibiotics since we have observed a rather extensive metabolism of both adriamycin and daunorubicin *in vivo* and *in vitro*.

Proposed Course:

Comparative enzymology of daunorubicin reductase from tissue sources in animals and in humans will be carried out, since this enzyme is apparently a most important control point for both daunorubicin and adriamycin disposition. The isolated metabolites of adriamycin and daunorubicin are being assessed for their pharmacologic activity so that we can obtain an overall understanding of their structure-activity relationships.

Honors and Awards: None

Publications:

Felsted, R.L., Gee, M., and Bachur, N.R.: Rat Liver Daunorubicin Reductase: An Aldo-Keto Reductase. J. Biol. Chem. May 1974.

Serial No. NCI-6014
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Cellular Control Mechanisms Affecting Cell Growth and Differentiation

Previous Serial Number: NCI-6014

Principal Investigators:
Nicholas R. Bachur
Douglas R. LaBrecque
Richard D. Leavitt

Other Investigators: Ronald Felsted

Cooperating Units: None

Man Years (July 1, 1973 to June 30, 1974)

Total:	2.2
Professional:	2.2
Other:	0

Project Description:

Objectives:

To study the control mechanisms which affect cellular growth and differentiation. These studies are aimed at elucidating control principles effecting all levels of cellular machinery as well as various levels of physiological control of the organized functioning animal. To determine differences in controls between differentiated and nondifferentiated tissues and between malignant and normal tissues. To study the effects of control changes on physiologic processes.

Major Findings:

In our studies on factors affecting cell growth and differentiation, we have concentrated on the importance of techniques for the isolation of liver parenchymal cells. The endogenous respiration of isolated rat liver parenchymal cells prepared by two enzymatic techniques was measured. Despite the production of a high percentage of viable cells by both methods as measured by the exclusion of trypan blue, the cells obtained by one of the techniques consume oxygen at only one-half the rate of those obtained by the other technique. It is suggested that the lower rate is the result of an insufficient oxygen supply and a lack of control of the pH of the incubation medium during the preparation of the cells. Isolated liver parenchymal cells are used to assess the mechanism of action of hepatic stimulator substance and for studies of drug metabolism.

In our studies on the cell-cell interaction and the mechanism of action of agglutinating substance, we are developing simple assays for agglutinating antibodies, lectins, and their hapten inhibitors. The initial rate of agglutination was measured by changes in transmittance across a standard cell suspension, and was found to be proportional to the concentration of agglutinin. By using the standardized technique, we have observed that cells from different species or individuals have different agglutination rates although they are inhibited with equal levels of agglutinin. Also, agglutinability of human erythrocytes increases during storage, under standard blood banking conditions. To further delineate the mechanism and the molecules responsible for agglutination, we have isolated and purified five isomitogens from phytohemagglutinin (PHA), of phaseolus vulgaris. We are studying the biological properties of these isomitogens.

Significance to Biomedical Research and Program of the Institute:

The isolation of materials from both plant and mammalian sources which effect the growth and differentiation of normal lymphocytes and hepatocytes is paramount in the study of the processes which control cellular activities. By using these purified materials as biochemical tools, we may be able to understand the biochemical mechanisms controlling cell proliferation and differentiation. Also, by investigating points where control of metabolic pathways are possible, we are approaching techniques where chemical agents may be used to control cellular growth, differentiation, and proliferation.

Proposed Course:

We will continue our studies on the control mechanisms which affect cellular growth and differentiation, and attempt to elucidate control principles affecting all levels of cellular machinery. Specifically, we hope to further purify our hepatic stimulator substance and to describe its physiological action in the normal animal. We hope to elucidate the mechanism of action of the isomitogens on cell membranes and determine how these materials cause mammalian cells to adhere to surfaces. Thirdly, we will continue our search for materials characteristic of altered metabolic pathways, which may be used to quantify malignant cells *in vivo*.

Honors and Awards: None

Publications:

LaBrecque, D.R., Bachur, N.R., Peterson, J.A., and Howard, R.B.: The Importance of Techniques Employed During the Enzymatic Preparation of Isolated Liver Parenchymal Cells. J. Cell. Physiol. 82: 397-400, 1973.

Belliveau, R.E. and Bachur, N.R.: Prostaglandin B Equivalents in Human Plasma. Prostaglandins 5: 241-248, 1974.

Fleisher, J.H., Marton, L.J., Bachur, N.R., and Mann-Kaplan, R.S.: Cholesterol in Cerebrospinal Fluid of Brain Tumor Patients. Life Sciences 13: 1517-1526, 1973.

Serial No. NCI-6016
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Pharmacodynamics of Cancer Chemotherapeutic Agents

Previous Serial Number: NCI-6016

Principal Investigator: Nicholas R. Bachur

Other Investigators: M. Steele
M. Goodman
M. Bessman

Cooperatings Units: Department of Biology, McCollum Pratt Institute,
The Johns Hopkins University

Man Years (July 1, 1973 to June 30, 1974)

Total:	0.7
Professional:	0.2
Other:	0.5

Project Description:

Objectives:

To investigate the biochemical and physiological effects of anticancer drugs and their mechanism of action. To correlate the effects and activities of the drugs with their chemical structures.

Major Findings:

The DNA polymerase induced by T4 bacteriophage catalyzes both addition of deoxynucleoside triphosphate to the 3'hydroxyl end of the primer, directed by DNA template, as well as the removal of nucleotide from this end. An interesting feature of the mutant DNA polymerases is that by comparison with a wild type enzyme, nuclease-to-polymerase ratios are higher for those from mutants carrying antimutator genes and lower for those from mutants carrying mutator genes. Another difference among these enzymes is their differential response to the antitumor drugs, adriamycin and daunorubicin, and to two other intercalating agents, ethidium bromide and 9-aminoacridine. At low drug concentrations the wild type and mutator DNA polymerases have stimulated incorporation rates of tritiated thymidine into DNA. As the drug concentration is increased, both enzymes are inhibited with the mutator enzyme showing less sensitivity. On the other hand, the antimutator DNA polymerase showed a higher sensitivity to each of the drugs and is inhibited significantly at drug concentrations where both wild type and mutator are stimulated.

The other catalytic function of these enzymes, their 3'exonuclease, is also inhibited by adriamycin and daunorubicin. However, the inhibition of nuclease is observed over the entire range of drug concentrations measured, and there is no region of nuclease stimulation nor is there any indication of differential inhibition of the nucleases associated with the three enzymes by either adriamycin or daunorubicin.

Significance to Biomedical Research and Program of the Institute:

In human cells adriamycin and daunorubicin increase error frequencies as measured by an increase in chromosomal aberrations. A DNA polymerase in human leukemic cells is mutagenic as compared to the enzymes in normal blastic cells, since the leukemic enzyme introduces more mismatched bases into DNA synthesis *in vitro*. These human leukemic cells are more sensitive to the anthracycline antibiotics than normal blood cells. Although mammalian DNA polymerases do not seem to have nuclease as a functional part of the polymerase molecule, it is likely that a separate nuclease or nucleases provide editing functions during DNA replication. If the anthracyclines inhibit exonucleolytic editing activity, we may expect the increased error frequency to be reflected in atypical cell types. An inhibition of their correcting capacity coupled with an already mutagenic DNA polymerase, may lead to high aberrant DNA molecules which could considerably reduce the survival of the malignant cell.

Another potential effect of the preferential stimulation of polymerase with inhibition of repair enzymes, (i.e., error removal), may be the accumulation of errors in the cellular DNA of normal tissues. The combination of these errors may result in a long term or chronic toxicity such as the myocardiopathy associated with both daunorubicin and adriamycin in humans. Finally, adriamycin has a greater inhibitory effect than daunorubicin against all of the polymerases tested. This observation correlates with clinical experience where adriamycin is a more effective drug than daunorubicin and with studies *in vitro* which show a higher calculated specific inhibitory activity for adriamycin.

Proposed Course:

Through the continued investigation of the pharmacodynamics of drugs we may be able to determine why these agents possess differences in their therapeutic index and spectrum of action.

Honors and Awards: None

Publications:

Goodman, M.F., Bessman, M.J., and Bachur, N.R.: Adriamycin and Daunorubicin Inhibition of Mutant T4 DNA Polymerases. Proc. Nat. Acad. Sci. April 1974.

Serial No. NCI-6241
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Clinical Pharmacology of Cancer Chemotherapeutic Agents

Previous Serial Number: NCI-6241

Principal Investigator: Nicholas R. Bachur

Other Investigators: Mark Green
Shigeru Takanashi
Charles Riggs Jr.
Peter H. Wiernik

Cooperating Units: Medicine Service, Baltimore Cancer Research Center, NCI

Man Years (July 1, 1973 to June 30, 1974)

Total:	1.8
Professional:	1.3
Other:	0.5

Project Description:

Objectives:

To study the disposition of cancer chemotherapeutic agents in humans with respect to absorption, distribution, biotransformation, and excretion. To characterize drug effects, potency, and maximum efficacy. To evaluate the toxicity of cancer chemotherapeutic agents in man and observe aspects of biological variations and other factors such as age, body weight, sex, route of adminstration, time of administration, rate of inactivation and excretion, tolerance, pathological state, genetic factors, and drug-drug interaction. To devise appropriate dosage schedules. To develop ancillary therapy to minimize toxic effects of drugs without effecting the therapeutic value.

Major Findings:

The previously reported efficacy of adriamycin was confirmed in our studies, and the efficacy of the intermittent single, high dosage schedule established. We have correlated clinical and pharmacologic observations in 96 patients treated with adriamycin. The basic dosage schedule was 60 mg/M^2 intravenously every three weeks. Pharmacokinetic studies showed a prolonged plasma half-life, low urinary excretion, and undetectable levels in the cerebrospinal fluid.

Patients with significantly impaired liver function had elevation and prolongation of plasma drug levels associated with severe toxicity unless their dosage was reduced by 50 to 75%. Of the 82 evaluable patients, 10 of 25 with sarcomas, 9 of 31 with carcinomas, and 15 of 26 with hematologic malignancies have achieved complete or partial remission. An additional 22 of 48 have improved. Six patients with solid tumors had progressive central nervous system disease while responding systemically. Adriamycin can be used with relative safety and high efficacy in a dosage schedule that was determined from our pharmacologic studies. Dosage reduction in patients with liver disease is essential to avoid life-threatening toxicity. This pharmacologic study of adriamycin in conjunction with the clinical trial has yielded information relevant to dosage scheduling; the necessity for dosage modification in patients with impaired liver function but not in those with impaired renal function, and the possible limitations of this highly active antineoplastic agent in the treatment of tumors of the central nervous system. The clinical trial has confirmed the efficacy of adriamycin in a broad spectrum of neoplastic disease including certain forms of lung cancer, as well as many sarcomas previously refractory to chemotherapy. Activity against such prevalent tumors as carcinoma of the breast and bronchogenic carcinoma is particularly noteworthy. In addition, the response rate of adriamycin in patients with malignant lymphomas, especially the less sensitive histiocytic and mixed lymphocytic histiocytic lymphomas appears to be higher than any single agent previously tested in a population already refractory to other chemotherapeutic agents.

Significance to Biomedical Research and Program of the Institute:

These studies have established and correlated clinical and pharmacologic observations for the scientific usage of adriamycin in malignant disease.

Proposed Course:

We will continue to study the clinical pharmacology of cancer chemotherapeutic agents and attempt to relate fundamental laboratory findings to clinical observations.

Honors and Awards: None

Publications:

Benjamin, R.S., Wiernik, P.H., and Bachur, N.R.: Adriamycin Chemotherapy—Efficacy, Safety, and Pharmacologic Basis of an Intermittent Single High-Dosage Schedule. Cancer. 33: 19-27, 1974.

Serial No. NCI-6210
1. BCRC, Lab. Pharm.
2. Enzymology and
   Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: The Development of Specific RNases as an Aid in the Sequencing of tRNA from Murine Leukemia Viruses

Principal Investigator: C. C. Levy

Cooperating Units : None

Man Years : (July 1, 1973, through June 30, 1974)
Total : 1/2
Professional: 1/2
Other : 0

Project Description:

Objectives

The isolation from microbial sources of nucleases which will hydrolyze long chain nucleic acids at specific base linkages as an aide in the sequential analysis of RNA from various murine leukemia viruses.

Major Findings

The technique of enrichment culture was employed to isolate a number microorganisms from soil which could degrade RNA at specific nucleotide bonds. Thus far nucleases specific for bonds containing cytidylic acid and uridylic acid have been obtained from two of the organisms. A third, Serratia sp., elaborates an enzyme which shows preferential specificity for adenylic acid linkages. The second of the two organisms described, Citrobacter sp., which hydrolyzes uridylic acid internucleotide bonds, has shown interesting alterations in its specificity under the influence of the polyamine, spermidine. The Citrobacter nuclease shows no activity towards polycytidylic acid until spermidine is introduced into the reaction mixture. At this point the degradation of cytidylic acid containing nucleotide bonds is rapid and complete. Of considerable interest also is that this is the first instance in which spermidine has been shown to have a direct effect on an enzyme. Of all the polyamines tested, the enzyme binds spermidine preferentially. The binding of the polyamine to the enzyme is the first case known where spermidine is bound to a protein. This binding has enabled us to isolate the peptide region of the enzyme to which the polyamine is bound. Two peptides have been isolated and the amino acid composition of both have been determined. It has been shown that for every mole of spermidine bound, one mole of peptide can be isolated.

Significance to Cancer Research

Nucleic acid metabolism, or, more correctly, the alteration of nucleic acid metabolism, is believed to play an important role in cancer induction. It is hoped that an understanding of the sequential relationship of one nucleic acid to the other in the construction of a large chain polymer in tumor-causing viruses may provide a clue to the nature of cancer induction. In addition, the effect of spermidine on the enzyme which hydrolyzes polyuridylic acid is of considerable interest. The reason is that in the urine of patients with diagnosed cancer there appears to be an increase in polyamine levels. The relationship of spermidine to the *Citrobacter* enzyme may have significance in terms of the physiological changes which are occurring in the cell as the tumor progresses. In addition, the ability of the protein to bind polyamines suggests that nucleases may play a considerably more pivotal role in the regulation of the cellular metabolism than had hitherto been suspected. This type of phenomena will be investigated in human enzymes as well, particularly in the enzymes that may be found in patients with cancer.

Proposed Course

The projected course of this study is to utilize these enzymes as a means of sequencing RNA isolated from tumor viruses. It is hoped that by establishing partial sequences in a number of RNA's from various tumor viruses, some relationship between the sequence and neoplasia will be seen.

Publications

Levy, C. C., Mitch, W. E. and Schmukler, M. Effect of polyamines on a ribonuclease which hydrolyzes RNA at uridylic acid residues. *J. Biol. Chem.* 248: 5712-5719, 1973.

Marotta, C. A., Levy, C. C., Weissman, S. M. and Varricchio, F. Preferred Sites of Digestion of a Ribonuclease from *Enterobacter sp.* in the Sequence Analysis of *Bacillus stearothermophilus* 5S Ribonucleic Acid. *Biochemistry* 12: 2901-2904, 1973.

Levy, C. C., Hieter, P. A. and LeGendre, S. M. Evidence for the Direct Binding of Polyamines to a Ribonuclease that Hydrolyzes Ribonucleic Acid at Uridylic Acid Residues. *J. Biol. Chem.*, in press.

Levy, C. C., Mitch, W. E. and Schmukler, M. Influence of polyamines on the hydrolysis of polynucleotides by *Citrobacter* nuclease. In *Polyamines in* Normal and Neoplastic Growth (Ed. D.H.Russell) Raven Press, New York, 1973, pp. 91-101.

Schmukler, M., Friedling, S. P. and Levy, C. C. Polyguanylic acid-inhibited ribonuclease of *Klebsiella*. II. Studies with synthetic polyribonucleotides. *Biochim. Biophys. Acta* 268: 403-410, 1972.

Friedling, S. P., Schmukler, M. and Levy, C. C. Polyguanylic acid-inhibited ribonuclease of Klebsiella. I. Purification and general properties. *Biochim. Biophys. Acta* 268: 391-402, 1972

Serial No. NCI-6242
1. BCRC, Lab. Pharm.
2. Enzymology and
   Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: Polyamines and Cancer; Functional Significance

Principal Investigator: T. P. Karpetsky

Other Investigators : C. C. Levy

Cooperating Units : Baltimore City Hospitals, Oncology Department

Man Years : (July 1, 1973, through June 30, 1974)
Total : 1/2
Professional: 1/2
Other : 0

Project Description:

Objectives
To define the functions of putrescine, spermidine, and spermine, and to use this information for cancer diagnosis and control.

Methods Employed
1. Determination of putrescine, spermidine, and spermine in urine of AML and ALL patients by use of an amino acid analyzer.
2. Correlation of levels of individual polyamines with state of disease and course of treatment as determined from patient medical records.

Major Findings
1. The use of a sensitive amino acid analyzer technique for measuring individual polyamine levels in the urine of cancer patients reaffirms the observation that these compounds are elevated in patients with cancer.
2. Putrescine levels appear to reflect both chemotherapy and state of disease.
3. Spermidine levels accurately reflect the state of disease, being elevated above normal control values prior to treatment, then falling to near normal values as remission of disease is obtained.
4. Spermine levels are always very low and these measurements are not useful indicators of state of disease or course of treatment.
5. Measurements of overall polyamine levels are misleading since the level of putrescine can change drastically when antibiotics are administered.

Significance to Cancer Research
The initial observation that polyamine levels increase in uncontrolled oncogenic states and decrease during remission of disease was proved valid. There is evidence to clearly indicate that these levels may be used as a clinical

tool for initial diagnosis of cancer patients and as an indicator showing when a remission has been obtained. Moreover, the interpretation of total polyamine levels and, in particular, putrescine levels, is open to question. The most reliable markers of disease state are the levels of spermidine.

Proposed Course

1. Further studies involving regular screening of AML and ALL patients with expansion into areas where remission is less marked or takes a longer time course (e.g., solid tumors of various types).
2. Establishment of the utility of a polyamine analyzer (Technicon) capable of using serum rather than urine, thus eliminating the problems inherent in 24-hour urine collection and enabling a more precise time course study of polyamine levels.
3. Initiation of studies of polyamines in states of rapidly proliferating human tissue; e.g., pregnancy, massive hepatic damage, etc., and comparison of these levels with those obtained from cancer patients with the aim of identifying possible false positives in the spermidine level test for cancer diagnosis.

Publications

Russell, D. H. Increased polyamine concentrations in the urine of human cancer patients. Nature 233: 144-145, 1971

Russell, D. H., Levy, C. C., Schimpff, S. C. and Hawk, I. A. Urinary polyamines in cancer patients. Cancer Res. 31: 1555-1558, 1971.

Russell, D. H., Levy, C. C. and Taylor, R. L. Increased DNA associated with rat liver nucleoli when isolated with spermidine. Biochem. Biophys. Res. Commun. 47: 212-215, 1972.

Russell, D. H. Polyamines in growth - normal and neoplastic. In Russell, D. H. (ed.) Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, pp. 1-13.

Marton, L. J., Graziano, K. D., Mardiney, Michael R., Jr., and Russell, D. H. Specific increases in polyamines in mixed lymphocyte reactions. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, pp. 215-219.

Gfeller, E., Levy, C. C. and Russell, D. H. Structural and biochemical changes in the nucleolus in response to polyamines. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, pp. 335-342.

Marton, L. J., Vaughn, J. G., Hawk, I. A., Levy, C. C. and Russell, D. H. Elevated polyamine levels in serum and urine of cancer patients: Detection by a rapid automated technique utilizing an amino acid analyzer. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, pp. 367-372.

Serial No. NCI-6245
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: Studies on Human Platelet Carbohydrate Metabolism: (1) The Effect of Cyclic AMP: (2) Interactions of Thrombin and Nucleotides and Their Effects on Glycolysis; (3) Effects of Storage and Various Drugs on Glycogenesis and Glycogenolysis

Principal Investigator: P. D. Zieve
M. Schmukler

Other Investigators : None

Cooperating Unit : Division of Hematology, Baltimore City Hospitals

Man Years : (July 1, 1973, through June 30, 1974
Total : 1
Professional : 1/2
Other : 1/2

Project Description:

Objectives
To study the metabolism of platelets in order to define the biochemical factors involved in platelet aggregation.

Main Findings
Washed human blood platelets are able to incorporate glucosamine and mannose into protein, thus producing glycoproteins. Cyanide and fluoride inhibit this synthesis but PGE, aspirin, con A, and nucleotides have no effect. Thrombin causes a marked decrease in incorporation which is probably the result of its proteolytic activity.

Significance to Cancer Research
Platelet aggregation is one of the factors involved in the clotting mechanism. Hemorrhage is one of the major problems in cancer chemotherapy and is often controlled by platelet transfusion. Studies of the biochemistry of platelet aggregation should contribute significantly to the intelligent storage and use of platelets in control of bleeding.

Proposed Course
These studies are being terminated.

Publication
Zieve, P. D. and Schmukler, M. The interaction of thrombin and nucleotides: Effects on glycolysis in human platelets. Biochim. Biophys. Acta 313: 350, 1973.

Serial No. NCI-6247
1. BCRC, Lab. Pharm.
2. Enzymology and
   Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title : Purification and Study of Bacterial and Mammalian Nucleases with Differing Residue Specificities

Previous Serial Number : Same

Principal Investigator : M. Schmukler

Other Investigators : C. C. Levy

Cooperating Units : None

Man Years : (July 1, 1973, through June 30, 1974)
Total : 1 1/4
Professional : 3/4
Other : 1/2

Project Description:

Objectives

1. The purification of nucleases with residue specificity for use in sequencing nucleic acids.
2. The study of the effects of various compounds upon these and other nucleases to delineate control mechanisms.
3. The study of nucleases in normal and cancer subjects with a view toward detecting differences which may be of diagnostic or mechanistic significance.

Major Findings

1. A ribonuclease with U specificity isolated from Enterobacter sp. was found to be affected profoundly by polyamines which caused stimulation of activity and altered residue specificity.
2. A nuclease purified from human plasma was found to have almost absolute specificity for intercytidylic acid bonds. This same nuclease is markedly elevated in multiple myeloma, so enzymes isolated from myeloma plasma and normal plasma were compared. Polyamines also stimulate the activity of this enzyme and apparently control its state of aggregation.

Significance for Cancer Research

The study of enzymes concerned with nucleic acid metabolism is essential to an understanding of the mechanisms of carcinogenesis and cancer control since there is obviously an alteration in nucleic acid metabolism in cancer and since most chemotherapeutic agents are directed in some way against nucleic acid metabolism. Nucleases which have some residue specificity should be valuable tools in the delineation of nucleic acid structure. It is also possible that nucleases with some type of specificity may be useful as chemotherapeutic agents. The studies with the polyribonucleotide inhibitors of nuclease activity may be useful in suggesting techniques whereby specific functions of malignant cells could be disrupted.

Nucleic acid metabolism is altered in malignancy. Study of enzymes involved in nucleic acid metabolism may offer clues as to the nature of the malignant process. These enzymes, also, may be indicators of certain types of malignancies.

Proposed Course

The human plasma ribonuclease will be studied in more detail in order to clarify the polyamine effect. Further studies will be made of nucleases from clinical material.

Publications

Levy, C. C., Mitch, W. E. and Schmukler, M. Influence of polyamines on the hydrolysis of polynucleotides by Citrobacter ribonuclease. In Russell, D. H. (ed.): Polyamines in Normal and Neoplastic Growth. New York, Raven Press, 1973, pp. 91-101.

Levy, C. C., Mitch. W. E. and Schmukler, M. Effect of polyamines on a ribonuclease which hydrolyzes ribonucleic acid at uridylic acid residues. J. Biol. Chem. 248: 5712-5719, 1973.

Serial No. NCI-6287
1. BCRC. Lab. Pharm.
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: Studies on Glycosidases of Human Platelets

Principal Investigators : M. Schmukler
P. D. Zieve

Other Investigators : None

Cooperating Unit : Division of Hematology, Baltimore City Hospitals

Man Years : (July 1, 1973, through June 30, 1974)
Total : 1 1/4
Professional : 3/4
Other : 1/2

Project Description:

Objectives

To study platelet glycosidases, their release reaction, and the relationship of these enzymes to platelet function and stability.

Major Findings

Concanavalin A, a lectin which causes blastogenesis of lymphocytes and aggregation of various malignant cells, produces a weak aggregation of human platelets but is an inhibitor of thrombin-induced platelet aggregation. Con A also causes release by the platelet of serotonin, adenine nucleotides, and the glycosidase, αmannosidase. The other glycosidases are not released by con A which blocks thrombin-induced release of these glycosidases.

Significance to Cancer Research

Platelet aggregation is one of the factors involved in the clotting mechanism. Hemorrhage is one of the major problems in cancer chemotherapy and is often controlled by platelet transfusion. Studies of the biochemistry of platelet aggregation should contribute significantly to the intelligent storage and use of platelets in control of bleeding.

Proposed Course

This study is being terminated.

Publication

Schmukler, M. and Zieve, P. D. The Effect of Concanavalin A on Human Platelets and Their Response to Thrombin. J. Lab. Clin. Med., 1974, in press.

Schmukler, M. and Zieve, P. D. Studies of Glycosidases in Fresh and Stored Platelets. J. Lab. Clin. Med. 80: 675, 1972.

Serial No. NCI-6288
1. BCRC, Lab. Pharm.
2. Enzymology and
   Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: Polyamine Biosynthesis in Rapidly Growing Tissues

Principal Investigator: D. H. Russell

Cooperating Units : None

Man Years : (July 1, 1973, through June 30, 1974)
Total : 1
Professional: 1
Other : 0

Project Description:

Objectives
To elucidate the pattern of synthesis and accumulation of pol
rapidly growing biological systems.

Methods Employed
1. Determination of putrescine, spermidine, and spermine in tissues by homogenization in acid, alkaline butanol extraction and high voltage electrophoresis for separation.
2. Ornithine decarboxylase activity (putrescine-forming enzyme) determined by liberation of $^{14}CO_2$ from ornithine-1-^{14}C.
3. S-adenosyl-L-methionine decarboxylase activity (spermidine-forming enzyme) determined by $^{14}CO_2$ release from S-adenosyl-L-methionine and from ^{14}C-spermidine formation from ^{14}C-putrescine; or from ^{14}C-spermine formation from ^{14}C-spermidine.
4. Routine determination of the relationships between RNA content and polyamine concentrations.

Major Findings
In mammary gland there is a very close correlation between polyamines and RNA content. In *Xenopus*, injection of putrescine directly stimulates RNA synthesis.

Significance to Cancer Research
An attempt is being made to elucidate the control process in rapidly growing cells. An understanding of this process, it is hoped, will gain understanding of the neoplastic process itself.

Proposed Course
This project is being terminated.

Publications

Russell, D. H. and McVicker, T. A. Polyamine metabolism in mouse liver after partial hepatectomy. Biochim. Biophys. Acta 244: 85-93, 1971

Russell, D. H. and McVicker, T. A. Polyamines in the developing rat and in supportive tissues. Biochim. Biophys. Acta 259: 247-258, 1972

Russell, D. H., Shiverick, K. T., Hamrell, B. B. and Alpert, N. R. Polyamine synthesis during initial phases of stress-induced cardiac hypertrophy. Amer. J. Physiol. 221: 1287-1291, 1971

Russell, D. H. Drug stimulation of putrescine and spermidine syntheses. Biochem. Pharmacol. 20: 3481-3491, 1971

Gfeller, E. and Russell, D. H. Autoradiographic distributions of ^{3}H-putrescine and ^{3}H-uridine in a Xenopus liver cell line. Z. Zellforsch 120: 321-331, 1971.

Russell, D. H. and McVicker, T. A. Polyamine biogenesis in the rat mammary gland during pregnancy and lactation. Biochem. J. 130: 71-76, 1972.

Manen, C.-A. and Russell, D. H. Polyamines in marine invertebrates. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, pp. 277-288.

Wylie, C. C. and Russell, D. H. The stimulation of RNA synthesis in mature amphibian oocytes by the micro-injection of putrescine. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, pp. 289-298.

Serial No. NCI-6289
1. BCRC, Lab. Pharm.
2. Enzymology and
   Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: Purification of S-adenosyl-L-methionine Decarboxylase from Rat Liver and from other Biological Sources

Principal Investigator: D. H. Russell

Other Investigators : C. C. Levy

Man Years : (July 1, 1973, through June 30, 1974)
Total : 1/4
Professional: 1/4
Other : 0

Project Description:

Objectives

1. To determine if there are separate decarboxylases for spermidine and spermine synthesis.
2. To determine if this system for polyamine biosynthesis is the same when isolated from widely divergent sources.

Methods Employed

1. S-adenosyl-L-methionine decarboxylase activity determined by $^{14}CO_2$ released from S-adenosyl-L-methionine when either putrescine or spermidine is used as a substrate. When putrescine is used as the substrate, there is spermidine formation. When spermidine is used as the substrate, there is spermine formation.
2. Sephadex gel filtration and fractionation.
3. DEAE-cellulose column chromatography.
4. Calcium hydroxylapatite column chromatography.
5. Affinity chromatography.
6. Protein determinations according to the method of Lowry et al.
7. Molecular weight determinations by the methods of Andrews and Martin and Ames.

Major Findings

S-adenosyl-L-methionine decarboxylase has been purified more than 350-fold from rat liver by a new procedure. It was found that 40% saturation with ammonium sulfate actually caused a loss of between 60-90% of enzyme activity. These results are in contrast to the effect of ammonium sulfate recently reported on the enzyme purified from ventral prostate. The molecular weight of the enzyme as determined by Sephadex gel filtration and by sucrose density centrifugation was found to be approximately 50,000. Enzyme activity was inhibited by sulfhydryl group inhibitors and by inhibitors of pyridoxal phosphate systems. Spermidine could be substituted for putrescine at all stages of purification, but there was always a 2:1 preference for putrescine. A similar type of enzyme has been isolated from sea urchins (L. pictus) and from L1210 cells grown in culture. The molecular weight of these enzymes ranges between 50,000-59,000, which seems very similar to that found for the liver system. The enzyme from sea urchins is inhibited by those substances affecting the rat liver system. At present electrophoresis is being used to determine if the enzyme will separate into subunits.

Significance to Cancer Research

Since the polyamine biosynthetic pathway is intimately tied with rapid growth, an understanding of the enzymes of the polyamine biosynthetic pathway would enable us to better elucidate inhibitors of the rapid growth process. With a purified enzyme system, one can test inhibitors that might be of value in cancer chemotherapy.

Proposed Course

This project is being terminated.

Publications

Feldman, M. J., Levy, C. C. and Russell, D. H. The purification of S-adenosyl-L-methionine decarboxylase from rat liver: Inability to separate decarboxylase from spermidine synthesis. Biochem. Biophys. Res. Commun. 44: 675-681, 1971

Feldman, M. J., Levy, C. C. and Russell, D. H. Purification and characterization of S-adenosyl-L-methionine decarboxylase from rat liver. Biochemistry 11: 671-677, 1971

Serial No. NCI-6291
1. BCRC, Lab. Pharm.
2. Enzymology and
   Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: Polyamine Metabolism in Spleens and Livers of Leukemic Mice. Effects of Potential Anticancer Agents

Principal Investigator: O. Heby

Other Investigators : D. H. Russell

Man Years : (July 1, 1973, through June 30, 1974)
Total : 1
Professional: 1
Other : 0

Project Description:

Objectives

To define the functions of putrescine, spermidine, and spermine, and to understand possible control mechanisms of their synthesis in leukemic mice.

Methods Employed

1. Spectrofluorometric quantitation of putrescine, spermidine, and spermine in tissues after dansylation and thin layer chromatography separation.
2. Ornithine decarboxylase activity (putrescine-forming enzyme) determined by liberation of $^{14}CO_2$ from ornithine-1-^{14}C.
3. S-adenosyl-L-methionine decarboxylase activity (spermidine- and spermine-forming enzyme) determined by $^{14}CO_2$ release from S-adenosyl-L-methionine (carboxyl-^{14}C).

Major Findings

A study conducted during the first months as a Visiting Fellow indicated that the ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities are elevated in spleens of leukemic mice 24 hours after intraperitoneal L_{1210} leukemia cell inoculation. The ornithine decarboxylase activity decreased by day 5 and was normal on day 8 of tumor growth, whereas the S-adenosyl-L-methionine decarboxylase activity remained elevated during the first eight days of tumor growth. The maximal survival of leukemic mice was nine days. It was shown that 5-azacytidine markedly suppressed the enzyme activities in leukemic mice when injected daily (day 1-9). The drug injections were started one day after tumor transplantation, in order to certify 100% tumor "takes." Except for the effects on the enzymes, the drug treatment caused a 300% increase in life span of the host mice. At the time when the drug treatment was interrupted, ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities increased rapidly both in drug-treated leukemic mice and in drug-treated control mice, indicating that 5-azacytidine exerts a general suppression on polyamine-synthesizing enzymes.

Significance to Cancer Research

An understanding of the early events that occur upon an invasion of malignant cells is necessary in order to structure effective chemotherapy.

Proposed Course

This study is being terminated.

Publications

Russell, D. H. Effects of Methotrexate and Cytosine Arabinoside on Polyamine Metabolism in a Mouse L_{1210} Leukemia. Cancer Res. 32: 2459-2462, 1972.

Heby, O. and Russell, D. H. Depression of Polyamine Synthesis in L_{1210} Leukemic Mice during Treatment with a Potent Antileukemic Agent, 5-azacytidine. Cancer Res. 33: 159-165, 1973.

Heby, O. and Russell, D. H. Changes in PolyamineMetabolism in Tumor Cells and Host Tissues during Tumor Growth and After Treatment with various Anticancer Agents. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, pp. 221-237.

Serial No. NCI-6902
1. BCRC, Lab. Pharm.
2. Enzymology and
   Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: The Characterization of Viral RNA Isolated from the AKR Murine Leukemia Virus

Principal Investigator: J. J. Frank

Other Investigator : C. C. Levy

Man Years	:	(July 1, 1973, through June 30, 1974)
Total	:	1/4
Professional	:	1/4
Other	:	0

Project Description:

Objectives

To isolate RNA in its undegraded form from an AKR murine leukemia virus so that the 5' and 3' termini of the RNA can be elucidated. The specific objective is to determine the polarity with which a message contained in the RNA is read on the mouse ribosome.

Major Findings

Thus far, the isolation of undegraded viral RNA has proved of considerable difficulty. A technique which has given some success is one in which the virus is disrupted by a combination of phenol, sodium dodecal sulfate and salt solution. The viral RNA is extracted into the aqueous phase and then subjected to gel electrophoresis. After the electrophoresis, the viral RNA is removed from the gel and subjected to alkaline phosphatase treatment. The phosphate at the 3' and 5' termini are removed and the RNA at the 5' end phosphorylated with polynucleotide kinase and ATP^{32}. The 5' terminus of the RNA is then released by digestion with alkali to give the 3'-5' nucleotide diphosphates. Analysis by paper chromatography and high voltage paper electrophoresis against standards made from the 3' nucleotide phosphates has shown that adenylic acid is the nucleotide at the 5' end of the RNA. The 3' terminus was identified after alkaline phosphatase treatment by oxidation of the terminal sugar residue to the dialdehyde followed by reduction to the trialcohol with tritiated $NaBH_4$. Two-dimensional thin layer chromatography of the alkaline digest of labeled RNA indicated the 3' terminus was uridylic acid. The viral RNA has also been hydrolyzed with base specific ribonucleases. Preliminary studies have shown that one enzyme can degrade viral RNA into oligonucleotides with chain links approximately 10-20 nucleotides in length.

Significance to Cancer Research

An understanding of the primary sequence of the viral RNA should be helpful in the elucidation of the mechanism by which the virus may replicate itself. Thus if that portion of the viral RNA responsible for the enzyme "reverse transcriptase" is known, then it may be possible to design drugs which can destroy it.

Proposed Course

The projected course is to continue the analytical studies and establish the polarity by which the RNA is read on the mouse ribosome.

Publications - None

Serial No. NCI-6903
1. BCRC, Lab. Pharm.
2. Enzymology and
   Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: Development of Specific DNases as an Aid in the Sequencing of DNA from Various Viral Tumors

Principal Investigator: C. C. Levy

Man Years : (July 1, 1973, through June 30, 1974)
Total : 1/2
Professional : 1/2
Other : 0

Project Description:

Objectives

The isolation from microbial sources of a DNase which will hydrolyze deoxyribonucleic acid at a specific base linkage as an aid in the sequential analysis of DNA obtained from a tumor virus.

Major Findings

The technique of enrichment culture was employed to isolate DNases from a microorganism which degrades DNA at specific nucleotide linkages. The organism has been classified as a Flavobacter and the enzyme has been characterized to some degree. The enzyme has a molecular weight of at least 200,000 and presumably breaks up into four, perhaps six, subunits. The enzyme appears to be an endonuclease in that alteration of the termini of various substrates has no effect on enzyme activity. The enzyme has strong specificity for deoxyadenylic acid, since it will not attack ribonucleic acid or polyribonucleotides under any conditions. The enzyme should be helpful in elucidating the primary sequence of DNA from tumor viruses. The enzyme, purified 2000-fold, will bind the polyamines, particularly spermidine. The binding site on which the polyamine adheres to the protein has been found to be a nucleophilic center. Thus this finding indicates that not only RNases but also DNases can bind polyamines. The binding site, at least with respect to the amino acid composition, appears to be generally similar. The significance of this finding is currently being explored.

Significance to Cancer Research

The ability to degrade deoxyribonucleic acid at specific linkages when the nucleic acid is obtained from viral sources should be of intense interest in sequencing the DNA of a tumor virus. It is hoped to compare regions of the DNA with comparable areas isolated from RNA tumor viruses.

Proposed Course
To isolate a homogeneous DNA from viral sources and to attempt a sequential analysis of the nucleotide composition. An attempt will be made to sequence the peptide isolated which binds spermidine and see the significance of this binding site with respect to the binding site on the ribonucleases. Since at the same site large ordered polynucleotides (poly AU) bind to the protein, it is of interest to determine if this binding site is the same and, if the same, is this a general phenomena throughout biological proteins? Would, for example, the binding site to which poly AU adheres be the same on a membrane surface, a surface which is of considerable importance in human chemistry?

Publications - None

Serial No. NCI-6925
1. BCRC, Lab. Pharm.
2. Enzymology and
   Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: Studies on the metabolism, function, and storage of human blood platelets

Previous Serial Number : NCI-6287

Principal Investigators: M. Schmukler
P. D. Zieve

Cooperating Units : Division of Hematology, Baltimore City Hospitals

Man Years : (July 1, 1973, through June 30, 1974)
Total : 1 1/4
Professional : 3/4
Other : 1/2

Project Description:

Objectives
To study the mechanisms of platelet aggregation and the platelet release reaction and the effect of various metabolic and pharmacologic factors upon these reactions. Also, to study the effects of storage upon platelets and to find techniques to preserve platelet viability and function during storage.

Major Findings
Concanavalin A, a lectin which causes platelet aggregation and release, binds to platelets. This binding is not affected by storage but is decreased by certain compounds which inhibit con A activity on the platelet. Con A also stimulates certain metabolic reactions of platelets.

Significance to Cancer Research
Discovery of the mechanisms of platelet action will lead to more effective use of platelet transfusions in the control of bleeding in cancer patients receiving chemotherapy. More effective means of platelet storage will help alleviate the scarcity problems one encounters when only fresh platelets can be used for transfusions.

Proposed Course
Binding and metabolic studies with con A will be continued. Certain chemotherapeutic drugs which affect platelet function will be studied.

Publications - None

Serial No. NCI-6926
1. BCRC, Lab. Pharm.
2. Enzymology and
   Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: Role of Polyamines in Regulating Activity and Specificity of Various Nucleases

Principal Investigator: T. P. Karpetsky

Other Investigator : C. C. Levy

Man Years	:	(July 1, 1973, through June 30, 1974)
Total	:	1/2
Professional	:	1/2
Other	:	0

Project Description:

Objectives

To establish the mechanism by which putrescine, spermidine, and spermine regulate the enzymatic activity and substrate specificity of nucleases.

Methods Employed

1. Nuclease activity determined spectrophotometrically after cleavage of a polynucleotide substrate under various reaction conditions.
2. Binding of polyamines to nucleases examined by means of equilibrium dialysis and equilibrium flow dialysis.
3. Computer models for the binding of polyamines to nucleases generated using a Wang 700 computer.
4. Determination of the relationships between polyamine binding and regulation of enzymatic activity and specificity.
5. Determination of minimum structural requirements for enzymatic regulatory function by use of model polyamines.

Major Findings

1. Enzymatic activity is strongly temperature-dependent in nucleases, the half-life of some of these enzymes being as short as 14 minutes at 37°.
2. These half-lives can be greatly extended, i.e. the enzyme protected by administration of one of the polyamines prior to the incubation at 37°.
3. Once enzymatic activity is lost by incubation at 37°, it can be restored by addition of one of the polyamines.
4. On the basis of kinetic evidence, spermidine appears to occupy at least two sites on one of these enzymes and can finely regulate the regeneration of enzymatic activity over a very limited range of spermidine concentrations.
5. Binding studies indicate that, although the polyamines bind to such proteins as bovine serum albumin, these compounds are at least an order of magnitude

more tightly bound to those nucleases whose activity is moderated by these amines.

Significance to Cancer Research

The reason for large increases in polyamine levels in various oncogenic states has been a matter for much speculation. The stimulation of activity and change in specificity of nucleases with concomitant production of a pool of nucleotides has been shown to be moderated by polyamines. The possibility that the polyamines act as concentration-dependent allosteric regulators of nucleases is in early stages of confirmation. The utility of the products of the nucleolytic reaction with respect to DNA and RNA building in cancerous tissue remains to be proven.

Proposed Course

1. Further studies on the effects of the polyamines and model compounds on nuclease activity by both the kinetic and binding studies.
2. Studies on the effects of the polyamines on DNA and RNA building enzymes isolated from normal and oncogenic sources.
3. Development of computer models for complex kinetic and binding situations found in the above studies.

Publications - None

Serial No. NCI-6927
1. BCRC, Lab. Pharm.
2. Enzymology and
Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: The Properties of Enterobacter Nuclease and its Interaction with the Polyamines

Principal Investigator: J. J. Frank

Other Investigator : C. C. Levy

Man Years : (July 1, 1973, through June 30, 1974)
Total : 1/2
Professional : 1/2
Other : 0

Project Description:

Objectives

To isolate a nuclease which can hydrolyze phosphodiester bonds at particular residues in RNA and would therefore be useful in determining the primary sequence of various types of ribonucleic acid. Further, it was of interest to determine the interaction of the ribonuclease with the polyamines which have been shown to bind to and affect the activity of Citrobacter nuclease.

Major Findings

The C ribonuclease was isolated from Enterobacter sp. which had been obtained from soil, using enrichment culture techniques. After lysing of the cells and preliminary purification of the nuclease by protamine and ammonium sulfate precipitations, the enzyme was purified using DEAE-cellulose chromatography followed by either a heat step or a putrescine affinity column. The C ribonuclease was shown to be homogeneous by polyacrylamide gel electrophoresis and its molecular weight (26,000) was determined by both sucrose density centrifugation and Sephadex gel filtration techniques. The nuclease was shown to digest only poly (C) and not poly (A), poly (U) or poly (G). Digestion of RNA indicated that the C ribonuclease cleaves between the 3'-phosphoryl moiety of cytidylic acid and the 5'-hydroxyl group of adenylic acid. The pH maximum for enzyme activity in either tris-maleate or Tris-HCl buffers was between 7.0 and 8.0.

Studies with the polyamines have shown dramatic increases in enzyme activity for assays run at low ionic strength while only modest enhancement of activity was observed at high ionic strength. The specificity of the nuclease was unaffected by the polyamines with either synthetic nucleotides or RNA as substrate. Inhibition of the enzyme by the ordered polynucleotides was reversed using spermidine or spermine, while other cations tested had no effect. The

C ribonuclease was shown to bind to the polyamines and to polyguanylic acid. Tryptic digestion of the poly (G)-bound enzyme gave peptides bound to poly (G) which were released with excess spermidine. Three spermidine-bound peptides were isolated after ion exchange chromatography and the nucleophilic amino acids, serine, glutamic acid and aspartic acid were shown to be present in each peptide after amino acid analysis.

Significance to Cancer Research

The polyamines have been shown to be present in high concentrations in the urine of cancer patients. It is of great interest to determine how the polyamines affect the ribonucleases and their role in controlling the concentration of RNA in the cell. This information would be useful in controlling the growth rate of rapidly proliferating cells.

Proposed Course

The projected course is to determine more fully the nature of the binding of spermidine to the isolated peptides and to examine the universality of these peptides as binding sites for large ordered polynucleotide complexes.

Publications

Levy, C. C. and Goldman, P. Residue specificity of a ribonuclease which hydrolyzes polycytidylic acid. J. Biol. Chem. 245: 3257-3262, 1970

Levy, C. C. *Enterobacter* ribonuclease. II. A reexamination of the enzyme's specificity toward internucleotide linkages. Biochim. Biophys. Acta 246: 476-486, 1971

Marotta, C. A., Levy, C. C., Weissman, S. M. and Varricchio, F. Preferred sites of digestion of a ribonuclease from *Enterobacter* sp. in the sequence analysis of *Bacillus stearothermophilus* 5S ribonucleic acid. Biochemistry 12: 2901-2904, 1973

Frank, J. J., Hawk, I. A. and Levy, C. C. Isolation of spermidine-bound peptides from *Enterobacter* ribonuclease. Biochemistry (submitted).

Serial No.: NCI-6020

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mechanisms of Human Lymphocyte Reactivity to Non-Self

Previous Serial Number: SAME

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Kenneth D. Graziano, Ph.D.
Myles R. Desner, M.D.
J. Leonard Lichtenfeld, M.D.
John C. Ruckdeschel, M.D.
Carl C. Levy, Ph.D.
Morton Schmukler, M.D.

Cooperating Units: Section of Enzymology and Drug Metabolism

Man Years:

Total:	2
Professional:	1 1/2
Other:	1/2

Project Description:

Objectives:

1. To enhance the sensitivity of immunologic induced blastogenesis in vitro.
2. To study reactivity to viral determinants using rubella as a model.

Methods Employed:

Short-termed tissue cultures in which the proliferative response of cells in question were monitored by measuring the incorporation of tritiated thymidine.

Major Findings:

1. The effects of double stranded synthetic polyribonucleotides, polyadenylic and polyuridylic acid (Poly AU), on the tritiated thymidine incorporation of cultured leukocytes was further evaluated. It had been previously shown that this agent could amplify responsiveness to suboptimal concentrations of strong antigenic stimuli. Further parameters of this amplification were evaluated. The data obtained indicated that the optimum range of poly AU concentration for effective amplification of lymphocyte

Major Findings (continued):

responsiveness was more specific for the particular lymphocyte population than for the antigen inducing stimulation. When used above optimum concentration, poly AU has an inhibitory effect inversely proportional to the antigen concentration utilized.

2. Rubella virus inactivated with Beta Propriolactone was found to be a potent stimulus in initiating lymphocyte immune responsiveness. Characterization of lymphocyte responsiveness to rubella virus when compared to hemagglutination inhibition antibody indicated that the former assay (lymphocyte blastogenesis) was a more sensitive technique for determining previous exposure to this agent. This greater sensitivity occurs because lymphocyte responsiveness in this system is dependent upon cell memory and is not directly related to continued presence of antigen or circulating antibody in the host.

Relevance:

1. Amplification of this in vitro assay of cell-associated immune function has been increased significantly by the use of poly AU. Additional parameters defining those conditions in which poly AU amplification can be expected to occur will give the scientist as well as clinician a more sensitive assay for determining host responsiveness to a variety of antigens including putative tumor antigens. In addition, an agent which is capable of causing such significant immune adjuvant effect in vitro may be also an extremely potent agent in vivo. Continued studies concerning mechanism of action of this agent may have much broader application than previously anticipated.
2. The use of the rubella model to determine host responsiveness to this virus and its comparison to hemagglutination inhibition antibody titers within the same individual would suggest that lymphocyte blastogenesis in vitro may represent an extremely sensitive system in which previous exposure to virus (putative oncogenic) may be studied in man.

Proposed Course:

To evaluate the basic mechanisms relating to the initiation of lymphocyte responsiveness by poly AU. To follow such studies by in vivo experimentation. To continue studying those aspects of lymphocyte responsiveness which may be effective in determining host responsiveness to tumor and/or oncogenic virus.

Honors and Awards: None

Presentations:

Proceedings of the 8th International Congress of Chemotherapy, 1973.

Publications:

Smith, K.A., Chess, L., and Mardiney, M.R., Jr.: The Relationship Between

Rubella Hemagglutination Inhibition Antibody (HIA) and Rubella Induced In Vitro Lymphocyte Tritiated Thymidine Incorporation. Cellular Immunology 8:321-327, 1973.

Mardiney, M.R., Jr., and Wren, R.E.: A Current Methodology for Performing Murine Mixed Lymphocyte Reactions. Biomedicine 19:331-334, 1973.

Graziano, K.D., Levy, C.C., Schmukler, M., and Mardiney, M.R., Jr.: Parameters for Effective Use of Synthetic Double Stranded Polynucleotides in the Amplification of Immunologically Induced Lymphocyte Tritiated Thymidine Incorporation. Cellular Immunology, December, 1973.

Smith, K.A., Mardiney, M.R., Jr., and Chess, L.: The Relationship Between Rubella Hemagglutination Inhibition Antibody (HIA) and Rubella Induced Lymphocyte Responsiveness in Vitro. Proc. of the 8th International Congress of Chemotherapy, to be published, 1973, Editor - Prof. H. P. Kuemmerle.

Serial No.: NCI-6248

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: To Determine the Effect of Freezing on the Transformation of Lymphocytes in the Mixed Lymphocyte Reaction and in Response to Specific Antigens and PHA.

Previous Serial Number: SAME

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Keith Kalmbach
Myles R. Desner, M.D.
J. Leonard Lichtenfeld, M.D.

Cooperating Units: None

Man Years:

Total:	1/8
Professional:	1/16
Other:	1/16

Project Description:

Objectives:

Previous work has defined that proper freezing of viable lymphocytes can allow for storage and subsequent reconstitution which will allow these cells to be effective in determining sensitivity to specific antigen in vitro. When frozen stored lymphocytes obtained from a normal individual at various times are cultured simultaneously, a response which is quantitatively identical can be observed in all lymphocyte populations. This assay provides a powerful tool for determining changes of immune response capability in man. Continued work in automating this area in order that large numbers of cells can be handled optimally have been carried out.

Methods Employed:

1. Lymphocytes were frozen in 10% DMSO and there proliferative response to antigen measured by the incorporation of tritiated thymidine in vitro.
2. Methods for automating this system were evaluated.

Major Findings:

Previous work had allowed us to develop a freeze controller which was capable in establishing an electronic analog of the desired cooling slope, providing continuous temperature messurement over the specimen to be cooled, and adding vaporized liquid nitrogen at the required rate to keep the specimen temperature aligned with cooling slope analog. In continuing this work, we have developed an automated system for processing cells prior to freezing. This has added significantly to diminishing the variabilities involved in this system.

Application:

The current controller and automated system for handling lymphocytes will allow for studies of optimum freezing of all types of mammalian cells. In addition, these tools decrease the need for technical observation during the prefreeze and freezing period.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6249

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Search for Antigen-Antibody Complexes in Renal Glomeruli of Tumor Patients

Previous Serial Number: SAME

Principal Investigator: John C. Sutherland, M.D.

Other Investigators: Michael R. Mardiney, Jr., M.D.

Cooperating Units: Department of Pathology, USPHS Hospital, Baltimore, Maryland.

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

1. To determine the association of long-term viral disease and neoplasia in man.

Methods Employed:

Immune complex deposition was determined by fluorescent microscopy. The determination of immune complex deposition in the kidney was dependent upon the finding of both IgG and Beta 1C globulin in a segmental pattern in the glomerular basement membrane. Human kidney material was obtained both postmortem and by open renal biopsy. Subsequent to localization of immune complexes in any given kidney, assays were performed to determine the antigen (s) present in such a complex. Specifically, various antisera directed against viral agents putatively involved in neoplasia were utilized in this study.

Major Findings:

303 postmortem kidneys were examined by immunofluorescence for the presence of glomerular immune complex deposition. Immune complexes were found deposited

Major Findings (continued):

in the glomeruli of 21. The highest incidence of this deposition occurred in kidneys from patients with acute leukemia, 14.3%, and the lowest in kidneys from patients with solid tumors (1.6%). The patients exhibited no clinical evidence of glomerular nephritis and the kidneys were essentially normal by light microscopy although electron-dense subendothelial deposits were seen in 4 out of 8. Although previous studies have identified a mammalian oncornaviral antigen in the complexes of two kidneys both from patients with acute leukemia, the identity of most of the antigens remain unknown. The detection and analyses of these complexes provided an important means of detecting host response to known and unknown antigens that are not now possible by routine serological methods.

Relevance:

Continued investigation in the area of subclinical immune complex deposition in the kidneys of patients with neoplasia as well as a variety of other diseases affords a powerful parameter for evaluating host response to both known and unknown antigens. The kidney is now the focal point for the study of reactants present in such complexes. It is clear at this time that the identification of the antigens present in such complexes will contribute significantly to knowledge concerning the pathogenesis of a number of diseases of which neoplasia represents a broad group. As methodology improves, continued work in this area should afford even greater sensitivity in defining the presence of such complexes and in determining the antigens involved.

Proposed Course:

1. To continue to screen for antigen in these complexes.
2. To continue to elute antibody from these kidneys and test for specificity.
3. To continue screening patients for this type of injury.

Honors and Awards: None

Presentations: None

Publications:

Sutherland, J.C., and Mardiney, M.R., Jr.: Subclinical Immune Complex in the Glomeruli of Postmortem Kidneys. Amer. J. of Med., in press, 1974.

Serial No.: NCI-6274

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Production of Tumor Specific Antibody in a Xenogeneic Species Utilizing Blocking Antibody: The Characteristics of Reactivity with the Complement System.

Previous Serial Number: 6274

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Walter P. Drake
Eugene F. Cimino

Cooperating Units: None

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

The characteristics of production of tumor specific antibody in the xenogeneic host utilizing the blocking system has previously been reported (J. National Cancer Institute 49:1063-1070, 1972; Cancer Research 32:1521-1525, 1972). Continued studies concerning the effectiveness of such antisera both in vivo and in vitro have been carried out. The purpose of this study has been particularly to define the characteristics of antibody and complement interaction which may allow for tumor cell kill both in vivo and in vitro. Defects in the complement system in the host bearing tumor were also studied.

Methods Employed:

Standard assays for the evaluation of antibody cytotoxic activity in vitro were utilized. These are complement dependent systems. In addition, standard assays for hemolytic activity (complement) were also utilized.

Major Findings:

1.
 A. Total hemolytic complement levels were measured in the C57BL/6 mouse bearing either EL4 lymphoma or B16 melanoma. Complement was shown to be progressively depleted as the tumor burden increased. Administration of antitumor antibody also demonstrated complement activity.
 B. Complement levels in the AKR, C57BL/6 and DBA/2 mouse could be enhanced by the passive administration of guinea pig complement.
2. The study of complement activity by means of immune adherence as well as hemolytic activity in 3 strains of mice after intraperitoneal subcutaneous injection of 6 transplantable tumors indicated that host complement activity was depressed in all cases by tumor transfer. cluded that serotherapy of murine tumors using passively administered antitumor serum may be compromised by host complement insufficient to mediate tumor cytolysis in vivo.
3. Previously absorbed antitumor antibody was shown to cross react with normal tissue antigen in the presence of a complement source sufficient to mediate tumor cytolysis in vitro. Such cross reactivity did not occur in the absence of complement.
4. Passive administration of complement in vivo was shown to potentiate the activity of antitumor antibody.

Relevance:

The above work again defines some of the parameters of antibody complement interaction in vivo as well as in vitro upon which tumor cytolysis depends. The work defining the mediation of cross reactive binding of tumor specific antibody to normal cellular determinants is of unique importance since such a phenomenon in part may explain the lack of effectiveness of putatively tumor specific antibody when used in vivo to mediate cell kill. The prerequisite that complement have homology with the antibody source in mediating cell kill as well as the findings indicating that tumor bearing animals become deficient in complement activity are important in further characterizing those aspects of antibody use which may be effective in mediating cell kill in vivo.

Proposed Course:

1. To further utilize these approaches to define tumor specific antigens on neoplastic cells.
2. To continue to evaluate various parameters of achieving specific cytotoxic activity against the tumor cell in vitro and in vivo.
3. To extend this approach in defining tumor specific antigens on human neoplastic cells.

Honors and Awards: None

Presentations: None

Publications:

Drake, W.P., and Mardiney, M.R., Jr.: The Enhancement of Antitumor Antibody Binding to Cross-Reactive Normal Tissue Antigens. A Complement Mediated Effect. Transplantation 16:189-198, 1973.

Drake, W.P., Ungaro, P.C., and Mardiney, M.R., Jr.: The Measurement and Manipulation of Hemolytic Complement Levels in Tumor Bearing C57BL/6 Mice. Biomedicine 18:284-289, 1973.

Drake, W.P., and Mardiney, M.R., Jr.: Depression of Complement Activity in Three Strains of Tumor Bearing Mice After Tumor Transfer. International J. of Cancer 11:719, 1973.

Serial No.: NCI-6292

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immune Adjuvants

Previous Serial Number: 6292

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Walter P. Drake
John C. Sutherland, M.D.
Eugene F. Cimino

Cooperating Units: None

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

The present study was carried out to define the effect of the chronic administration of polyadenylic-polyuridylic acid in AKR mouse as both a preventive and therapeutic measure to inhibit or delay the onset of spontaneous leukemia in this strain. Previous in vitro work within this laboratory indicated that this synthetic ribonucleotide had a unique effect on the amplification of in vitro lymphocyte responsiveness. We felt this study of immune modulation on spontaneous leukemia in the AKR mouse was especially relevant since this animal has recently been shown to be immunologically responsive to the specific etiologic agent causing his leukemia.

Methods Employed:

Beginning at 7 weeks of age, AKR mice were treated with the synthetic double-stranded polynucleotide, polyadenylic-polyuridylic acid (Poly A:U). The effectiveness of this agent was compared to the simultaneous administration of saline.

Major Findings:

Of the various doses evaluated, 1500 micrograms poly A:U administered subcu-

Major Findings:(continued):

taneously weekly prolonged dramatically the long term survival of these mice, which have a genetic propensity to develop leukemia. At 9 1/2 months, when more than 50% (14/25) of saline control animals had succumbed to leukemia, 100 % (25/25) of poly A:U animals survived. Although all animals eventually died of leukemia, poly A:U shifted median survival time by 4 1/2 months over saline controls. This increased survival represents the most successful published treatment of this tumor to date.

The lack of toxicity of this agent plus evidence suggesting that more frequent administration at higher doses may result in more effective immune amplification suggest that poly A:U may elicit even greater longevity in the AKR model. This agent is of great potential importance as an immune adjuvant since its effectiveness has been amply described in vivo and in vitro. The lack of toxicity is of extreme importance.

Relevance:

Modulation of the immune response may represent an important aspect of the eventual control and cure of neoplastic disease. The present agent (poly A:U) is important because it represents a class of biological compounds whose mechanism of effectiveness may be the key to developing even more improved systems for control of the immune system.

Proposed Course:

To further evaluate this agent as well as other agents capable of enhancing host immunologic responsiveness.

Presentations:

American Association for Cancer Research, Houston, Texas, 1974

Publications:

Drake, W.P., Cimino, E.F., Mardiney, M.R., Jr., and Sutherland, J.C.: Prophylactic Immunotherapy of Spontaneous Leukemia of AKR Mice with Polyadenylic-Polyuridylic Acid (poly A:U). J. Natl. Cancer Inst., in press, March, 1974.

Serial No.: NCI-6250
1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ultrastructural and Tissue Culture Studies of Acute Leukemic Cells of Patients before and during treatment and donor granulocytes harvested for transfusion therapy of these patients.

Previous Serial Number: Same

Principal Investigator: Frances T. Sanel

Other Investigators: Peter H. Wiernik,
Joseph Aisner
Cooperating Units: Tumor Service

Man Years: Total: 1/4
Professional: 1/8
Other: 5/8

Project Description:

Objectives: Evaluation of suitability for transfusion(to neutropenic patients) of normal, granulocytes harvested and treated by various techniques by comparative studies of micromorphology, viability and function. Continuing studies are directed toward determining the responsiveness to and cytological aspects of phagocytosis-provoking agents such as thorium dioxide, and viable bacteria, on peripheral blood and bone marrow cells from patients with acute leukemia before and during treatment. Histochemical and cytochemical studies on this material as well as concommitant search for virus are continued.

Major Findings:
Normal donor granulocytes harvested by filtration leukopheresis were studied after elution from nylon fibres and were viable by dye exclusion and phagocytosed gram positive and negative bacteria adequately. Electron microscopy when compared with controls revealed diminution of primary and secondary lysosomal granules and cytoplasmic vacuolization when cells were suspended in ACD plasma.

Aberrant cytomorphology and deficiency of lysosomal enzymes in leukemic cells have been observed. Myeloperoxidase deficient leukemic granulocytes from a patient with myelomonocytic leukemia were replaced by a population of cells with normal amounts of this enzyme after chemotherapy. Although the ability to phagocytose test material was not impaired in the untreated state, lysosomal breakdown of ingested material was not observed.

Massive aggregates of microfilaments in leukemic cells from untreated patients and their reappearance in relapse after disappearance in remission have been noted in several cases.

Proposed Course:

1. Continued study of monocytic and myelomonocytic leukemias with phagocytosis provoking agents.

2. Continued studies of harvested granulocytes to correct functional, clinical and micromorphological defects by varying techniques of collection and subsequent treatment before transfusion into leukopenic patients.

3. Cytochemical study of the glycocalyx of leukemic cells and utilization of freeze-etch techniques to elucidate the "zipperlike junctions" fromed by some leukemic cells under test conditions.

4. Soft-agar tissue culture to induce differentiation of leukemic stem cells and responsiveness of these cells to chemotherapeutic agents.

5. Cytochemical study of microfilaments.

Signifiance to Cancer Research:

The development of procedures for processing harvested granulocytes and their evaluation in order to reconstitute viable functionally competent cells for transfusion will supply a valuable therapeutic adjunct to patients made leukopenic by their disease or treatments for their disease,who have developed infections refractory to antibiotic therapy.

Publications:

Aisner, J. and F. T. Sanel 1974 Ultrastructural and Functional Studies of granulocytes obtained by filtration leukopheresis. Proc. Amer. Assn. Cancer Res. Abstract #276.

Serial No.: NCI-6272
1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of Myelomonocytic Leukemia in an Animal Model System: Electron Microscopy and Biological Experiments

Previous Serial Number: NCI-6272

Principal Investigator: Frances T. Sanel

Other Investigators: None

Cooperating Units: Laboratory of Viral Diseases (Dr. Janet Hartlet)
Microbiological Associates (Dr. J. C. Parker)

Man Years (July 1, 1973 to June 30, 1974)

Total:	1/2
Professional:	1/8
Other:	3/8

Project Description:

Objectives:

To study etiology, passaging under varying conditions and trans-species grafting of transplantable, muramidase-producing myelomonocytic WEHI-3 tumor cells utilizing techniques of short term tissue culture, hematological, histological, histochemical and ultrastructural techniques.

Precise identification of the specific virus type within the murine Onrcona virus group, and further evaluation of their oncogenic capability.

To determine the phagocytic competence of myelomonocytic neoplastic cells whose normal counterparts actively ingest particulates.

Major Findings:

1. Balb/c Mice:

Muramidase-producing tumor cells contained peroxidase positive granules additionally confirming their identity as non-lymphoid precursors.

We identified "C" type viral particles by electron microscopy in tumor cells of spleen, liver, and bone marrow. They were tested as tumor extracts by complement fixation and XC mouse embryo cytopathogenicity tests and they contained the group specific antigen (gs) of murine leukemia viruses and demonstrated high levels of infectivity. Recent results of cell-free passage into newborn Balb/c mice reveal massive production of "C" type viruses after a four month latency period. The disease in these animals differing from that in donor mice still remains to be characterized since the majority of mice treated with cell-free filtrates developed lymphatic leukemia, lymphoma, and/or hind leg paralysis and in a few instances, massive liposarcomas containing C particles, with no evidence of lymphomatous disease.

Investigation of hind leg paralysis revealed considerable osteosclerosis with occlusion of intervertebral foramena. In a number of animals, pericardial deposits were observed, principally on atrial surfaces and were identified by histochemical and ultrastructural studies as calcium. Serum or plasma calcium levels were within normal limits. Significance of these deposits present in some normal controls as well is being investigated.

Preliminary experiments testing transplacental transmission of viruses harvested from WEHI-3 tumors encourage further investigation.

In several cell transplant generations, unusual rodlike structures were observed in tumor cells. These were continuous with the rough endoplasmic reticulum and occasionally were formed on the same cistern that demonstrated budding "A" type particles in another area. We speculate that these structures may be a cellular response to viral infection, may be a manifestation of neoplastic transformation, or that they may represent aberrant viral precursor elements.

2. Trans-Species Studies:

Trans-species passage of Balb/c tumor cells into newborn inbred Fisher rats resulted in induction of a transplantable lymphoid leukemia with massive thymomas, mesenteric tumors, enlarged spleens and systemic dissemination of leukemic cells. "C" type viral particles in tumor extracts examined by complement fixation and XC tests contained the gs antigen of MuLV and also demonstrated very high titers of infectivity.

Transfer of tumor cells from Fisher rats into newborn Balb/c mice induced lymphoid neoplasia in the latter.

Significance to Cancer Research:

These animals systems could be valuable models for the study of etiology, diagnosis and therapy of human neoplasia.

Proposed Course:

1. Continued study of the oncogenic potential of the "C" type particles.
2. Further study of the diseases in Balb/c mice and Fisher rats induced by cell-free filtrates of the WEHI-3 tumor.

3. Experiments to test transplacental transmission of WEHI-3 tumor cells or viruses in pregnant Balb/c mice.
4. Therapeutic studies on the WEHI-3 Balb/c animals in collaboration with Dr. M. Walker and Dr. P. Wiernik.
5. Freeze-etch studies of virus producing cells.
6. Soft-agar tissue culture of WEHI-3 tumor cells directed toward differentiation, virus production and responsiveness to chemotherapeutic agents.
7. Further study of the unusual cytoplasmic rodlets.

Publications:

Sanel, F.F. Studies of Myelomonocytic Cells in Balb/c Mice Producing Infectious "C" Type Viruses. Cancer Res. 33: 672, 1973.

Serial No.: NCI-6923
1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Fine Structure and Cytochemistry of Human Neoplastic Solid Tumors.

Principal Investigator: Frances T. Sanel

Other Investigators: Arthur Schantz

Cooperating Units: Department of Pathology

Man Years: Total: 1/8
Professional: 1/8
Other: 3/4

Project Description:

Objectives:
To study with the collaboration of the consulting pathologist, surgical specimens of solid tumors that are difficult to diagnose by conventional histochemical methods and/or those not previously described in the literature.

Major findings:
In the present study of a surgical specimen of a solid tumor,a diagnosis of undifferentiated nasopharyngeal carcinoma of entodermal origin was made on the basis of ultrastructural and cytochemical studies differentiating the tumor cells from those of possible chordoma.

Significance to Cancer Research:
Such studies that describe, characterize and identify aberrant neoplastic cells are an aid to prognosis and treatment.

Serial No.: NCI 6924
1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ultrastructural Studies of Aberrant Cells in Possible Preleukemic States.

Previous Serial Number: None

Principal Investigator: Frances T. Sanel
J. Breton-Gorius

Other Investigators: G. Flandrin, M. Y.Daniel, J. Chevalier, M. Lebeau

Cooperating Units: Institute de Recherches Sur Les Maladies du Sang, Creteil and Paris, France

Man Years (July 1, 1973 to June 30, 1974)

Total:	1/8
Professional:	1/8
Other:	3/4

Project Description:

Objectives:

In the present investigation to study and characterize the abnormal erythroid precursors from the bone marrow of a patient with refractory anemia and ineffective erythropoiesis.

Major Findings: Ultrastructural studies in our laboratories of material sent to us from France, revealed unusual junctional complexes between aberrant mono-and multinucleated erythroid precursors and similar seemingly intracellular structures resembling the zipper-like junctions we reported (Science 168, 1970) in human leukemic monocytes.

Significance to Cancer Research:
These studies may elicit predictive factors toward definitive diagnosis that would aid in prognosis and treatment regimens.

Proposed Course:
1. Persistent study at the electron microscopic level of blood cells from patients exhibiting unusual hematologic dyscrasias to determine common cytologic characteristics during the course of the disease.

Scientific Exhibit:
Bizarre Septate Junctions in human erythroblasts: Membrane pathology in a case of refractory anemia. Washington Soc. Electron Microscopy 1973.

ANNUAL REPORT

OFFICE OF THE ASSOCIATE DIRECTOR FOR CANCER THERAPY EVALUATION

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Cancer Therapy Evaluation Program has two major functions within the Division of Cancer Treatment (DCT). The first is the coordination and monitoring of all clinical trials performed with new anticancer agents sponsored by the DCT Drug Development Program. This is accomplished by the Investigational Drug Branch (Milan Slavik, M. D., Ph. D., Acting Chief) and its Drug Liaison and Distribution Section (John Penta, Ph. D., Head) which provides liaison with Food and Drug Administration and is responsible for the distribution of investigational drugs throughout the world.

The second major function encompasses the development, coordination, and monitoring of combined modality approaches to cancer treatment. In this area, the Combined Modality Branch (Stephen K. Carter, M. D., Chief) has the responsibility of developing extramural disease-oriented treatment programs with combined modality approaches. In addition, the Branch attempts to monitor the various disease-oriented treatment thrusts that the NCI sponsors both within and outside the DCT, as well as ongoing international programs.

In considering the expanded functions and responsibilities of the DCT under the National Cancer Program, one of the most serious problems is the conflict between the clinical resources needed by the Drug Development Program and the exigencies of devising an optimal combined modality treatment strategy for solid tumors. This conflict is most critical in the allocation of clinical resources in the advanced disease states of different tumors. For each tumor, phase II resources may be used for investigating single agents, combination chemotherapy regimens, or even combined modality probes (e. g., drugs plus immunotherapy or drugs plus radiotherapy). Single agents include established drugs that have not been tested against a given tumor, new schedules of established drugs, or investigational drugs. Investigational drugs may be those with some measure of established activity (e. g., nitrosoureas, adriamycin) or new drugs for which Phase I study has recently been completed.

Each tumor must be analyzed from the standpoint of what is required to develop an overall strategy for the specific treatment (Figure 1). The drugs or regimens that are chosen for Phase II and III study in patients with advanced disease must integrate into this strategy. However, the strategy may, in many cases, directly conflict with the needs of a drug development program which places as many as 10 new drugs each year into clinical trial.

Currently, there are approximately 25 commercially available standard antitumor agents. The Drug Liaison and Distribution Section holds INDs for more than 50 anticancer drugs, many of which show some evidence of anticancer activity. The program currently has 10 drugs in Phase I study and, therefore, approximately 40 drugs that have passed Decision Network (DN) 2 in the linear

Considerations		Clinical Trial Choices
1. Cure rate with local and regional therapy 2. Cell kill potential of chemotherapy	Primary treatment of local and regional disease	Phase IV Surgery and/or x-ray plus chemotherapy and/or immunotherapy
1. The number of active drugs and their mechanisms of action, toxicity patterns, and pharmacology 2. The potential of radiotherapy and immunotherapy.	1st Recurrence "Early" disseminated disease	Phase III 1. Determinatin of optimum combination 2. Testing of combined modality approach 3. Determination of optimum single drug 4. Elucidation of optimum schedule of active drug
1. Number of standard drugs tested and results. 2. Available data on investigational drugs 3. Active drugs of potential for combinations a. Mechanisms of action b. Toxic effects c. Pharmacology	2nd Recurrence "Advanced" disseminated disease	Phase II 1. Standard drugs not tested 2. New investigational drugs 3. New combinations 4. "Active" drugs used on new schedules

Figure 1. Idealized flow of therapy in solid tumors with factors for consideration and clinical trial choices.

array and are progressing toward eventual clinical trial. Since the input to the screen is doubling, even the use of all the currently available clinical resources would be inadequate to meet the needs of the drug development linear array. Obviously, all the clinicall resources cannot be used in this way and it is essential that an overall strategy be designed for using these precious resources. The DCT must make decisions in two critical areas:

(1) How many of the major solid tumor types will be included in the program and what priority should they have among themselves and in relation to ongoing programs in leukema and lymphoma?

(2) How should the DCT allocate advanced disease resources, within the major solid tumors, between Phase II studies of new investigational drugs and other single agents or combination regimens? As a result of this kind of analysis, a decision must be reached on the number of new drugs arising from the drug development pool that can be handled. Lastly, and most difficult, is the question of how many drugs are actually best within the DCT overall treatment plan.

During the past year, the CTEP staff has studied some approaches to the problem of allocating advanced disease resources according to priorities established among the major tumor types. This is based on an analysis of data obtained in the clinical evaluation of 29 drugs, each of which has proven definitely active against at least one tumor type from among the hematologic malignancies or solid tumors.

Four solid tumors for which program seems absolutely essential because of their incidence and mortality are bronchogenic carcinoma, adenocarcinoma of the breast, large bowel carcinoma, and pancreatic carcinoma. These tumors constitute Group I - the highest priority for study.

A. Bronchogenic Carcinoma

In lung cancer, the data for 25 of the 29 anticancer drugs is sufficiently reliable for some evaluation of activity. Selawry's review of monochemotherapy highlights the greater gaps that exist when the data are viewed on the basis of individual cell type. Nevertheless, for many years the single agent chemotherapy of lung cancer has been pursued at a more aggressive level than in any other solid tumor.

Results, with the exception of small cell anaplastic tumors, have been dismal. Although objective tumor regression is observed in a reasonable number of cases with all cell types, the impact on survival is minimal or non-existent. Combination chemotherapy in cell types other than small cell anaplastic has only slightly increased the regression rate and survival. Chemotherapy combined with radiotherapy has been almost universally a failure in spite of vigorous approaches in the last 10 years. Even in patients having small cell anaplastic lesions, with regression rates over 50% and survival doubled in the best studies, this has meant only several additional months of life.

For this tumor, the discovery and development of new active drugs might hold the highest priority. Phase II studies of new agents or previously untested standard agents are more needed than Phase III trials of drug combinations or drugs plus radiotherapy. Priority areas of investigation for advanced disease that is not amenable to X-ray or surgery might be:

1. New investigational drugs
2. Standard drugs, which are totally untested; filling in knowledge gaps by cell type for drugs that basically have not shown great activity in less defined trials (e.g., 5-FU should be at the bottom of this priority level).
3. New combinations of "active" drugs.
4. "Active" drugs on new schedules.

B. Adenocarcinoma of the Breast

Most of the standard agents have been adequately evaluated. This tumor has been highly responsive to a wide range of drugs and drug combinations have been successful. The greatest need is to evolve the optimum combination of the various active agents.

It is no longer possible, or even desirable, to test every new drug that comes into clinical trial because advanced disease resources are heavily committed to combination studies. Patients failing to respond to combination chemotherapy will eventually be available for new drug studies. Suggested priorities are:

1. Combination regimens.
2. Established drugs that have not been adequately tested.
3. New investigational drugs.
4. Active drugs on new schedules.

C. Large Bowel Carcinoma

Most of the standard drugs have been adequately studied but results have been extremely poor and no single drug exceeds the 20% regression rate of 5-fluorouracil. Drug combinations have shown only nominal success, possibly reflecting the weakness of the individual drugs. The greatest need is for new active agents among the priorites as follows:

1. New investigational drugs.
2. Standard agents not previouls tested.
3. Standard agents with hints of activity on untested schedules (e.g., methotrexate, melphalan).
4. Drug combinations.
5. Active drugs on untested schedules ("5-FU is 5-FU is 5-FU..... and Ftorafur too).

D. Pancreatic Carcinoma

This tumor presents the most dismal prognosis of any solid tumor with the possible exception of esophagus. The curative potential of surgery is so poor that prospects for successful combined modality approaches are grim. Earlier diagnosis should have the highest priority.

Only 4 of the 29 standard drugs have been adequately evaluated and there may may be considerable potential in drugs having established activity in other tumor types. There also is a need for uncovering new active agents.

The critical question is--do all the major gastrointestinal adenocarcinomas have the same general responsiveness, or lack of response, to chemotherapy? This appears to be true with 5-FU, but not with the nitrosoureas and it is questionable for mitomycin C. There is little potential for combination chemotherapy at this time because activity has been established for so few drugs. The suggested priorities for study are:

1. Standard agents not previously tested.
2. New investigational drugs.
3. Combinations.
4. Active drugs on untested schedules.

Nine other solid tumors are assigned to the next order of priority (Group II) as highly desirable for inclusion in the program because of either their incidence and mortality or the potential for success in therapy. The priorities for drug evaluation in these tumors are shown in Table 1.

A. Ovarian Cancer

This tumor is highly responsive to chemotherapy; optimal use of alkylating agents alone yields a 50% response rate. However, only 8 of the 29 standard agents have been adequately evaluated. Of these, 3 are definitely active and 3 show hints of activity. Standard agents that have never been tested, or only inadequately so, should be evaluated.

Combination regimens should be aggressvely studied. There is great potential for successful combined modality therapy in this tumor.

B. Stomach Cancer

Chemotherapy has barely been evaluated in this tumor which some rank high as a major killer. Only 3 of the 29 standard agents have been tested to any degree, and all (5-FU, mitomycin C, and BCNU) exhibit a low order of effectiveness.

Since BCNU + 5-FU has been evaluated with some success, and mitomycin C combines poorly, there is little that remains to be done with combinations. The greatest need is for testing of standard agents and new investigational drugs.

C. Squamous Cell Carcinoma of the Cervix

This tumor appears to be potentially responsive to chemotherapy. Nine of the 29 established agents have been tested; 3 are active and 4 show some evidence of activity.

Combinations have not been fully evaluated but the success of this approach seems promising since non-myelosuppressive drugs like vincristine and bleomycin show activity.

Table 1. Study priorities for major solid tumors and hematologic malignancies.

Tumor Type	Order of Priority for Study: New Investigational agent	Standard Drugs Not Previously Tested	Combinations	Active Drugs on Untested Schedules
SOLID TUMORS				
Ovarian Cancer	4	2	1	3
Stomach Cancer	2	1	3	4
Squamous Cell Carcinoma of Cervix	4	2	1	3
Squamous Cell Carcinoma of Head and Neck	4	2	1	3
Prostate Cancer	2	1	3[†]	
Testicular Carcinoma	3	2	1	4
Soft Tissue & Bone Sarcoma	4	2	1	3
Brain Tumors	3	1	2	4
Malignant Melanoma	1	2	3	4
Bladder Carcinoma	3	1	2[‡]	4
Renal Cell Carcinoma	2	1		
Esophagus	2	1		
Endometrial	2	1		
Hepatoma	2	1		
HEMATOLOGIC MALIGNANCIES				
ALL	2	1	3[§]	4
AML	1	2	3	4
CML	3	2	1	4
CLL	3	2	1	4
Multiple Myeloma	3	2	1	4
Hodgkin's Disease	3	2	1[‖]	4
Non-Hodgkin's Disease	3	2	1	4

[†] After chemotherapeutic "tools" are elucidated

[§] In advanced refractory disease

[‡] Adriamycin + 5-FU

[‖] Combinations not cross-resistant with MOPP

D. Squamous Cell Carcinoma of the Head and Neck

This is another tumor that apparently may respond to chemotherapy. In many studies, methotrexate has produced a 50% response rate. Only 6 of the 29 standard drugs have been evaluated, but 2 of these are active and 2 show evidence of activity. Combination chemotherapy studies are practically non-existent.

E. Prostate

Chemotherapy data are minimal or completely lacking and no non-hormonal agent has established activity. Thus, the tools for combination studies are nonexistent at this time.

F. Testicular Carcinoma

This tumor is highly responsive to chemotherapy and a curative potential (≃ 10%) exists in advanced metastatic disease. Ten of the 29 drugs have been tested and 9 show some degree of activity. One new investigational drug (platinum) has exhibited high activity.

Combinations have been used since the mid-1950s but their superiority over single agents is unestablished by controlled trials. A new combination (vinblastine + bleomycin) is reported to give a 90% response rate. Combinations should be exploited further. The potential for a successful combined modality thrust is excellent.

G. Soft Tissue and Bone Sarcomas

With the advent of adriamycin, responsiveness to chemotherapy has been uncovered. Only 6 of the standard drugs have been adequately tested but all of them show evidence of activity. The data are not extensive, especially regarding response on the basis of individual cell type with the exception of adriamycin.

Combination approaches are being aggressively pursued by various groups. Prospects are good for a combined modality attack, where embryonal rhabdomyosarcoma and Ewing's sarcoma already indicate the triumphs of such an approach.

H. Brain Tumors

Chemotherapy data for tumors of the central nervous system are largely limited to glioblastoma multiforme. Some responsiveness to drugs has been reported within the limitations imposed by the difficulties of evaluating objective regression. Ten of the 29 standard agents have been tested with 2 found active (both nitrosoureas) and 2 showing hints of activity.

With only a few exceptions, combinations have not been tested. A combined modality approach has shown success in terms of statistically significant improved survival, but nearly all patients still die within one year.

I. Malignant Melanoma

Chemotherapeutic agents have been extensively tried but results are generally poor. Twenty of the 29 agents have been tested and only 2 are active. The best drug (DTIC) is effective in only 20% of cases. The greatest need is for more active new drugs.

Combinations also have been extensively studied without success; response rate is increased only slightly, if at all. Immunotherapy appears to offer reasonable potential.

Finally, there are 5 additional solid tumors (Group III) for which programs should be developed provided resources exist. However, they are of lowest priority because of smaller incidence or lack of potential for success of combined modality approaches.

A. Bladder Carcinoma

Data that can be systemically used for a decision on activity are available for only 2 of the 29 standard drugs. Both of these (5-FU and adriamycin exhibit evidence of activity. Obviously, other standard agents should be evaluated because this tumor may be reasonably responsive based on the experience with only 2 drugs.

The only combination that is a reasonable candidate would be adriamycin + 5-FU.

B. Renal Cell carcinoma

Attempted chemotherapy has been a nearly total failure. Data is available on only 5 of the standard agents and none is active. There are no chemotherapeutic tools for combination studies.

C. Esophagus

Again, there is no active chemotherapy. Three standard drugs have been tested and none is active.

D. Endometrial

No chemotherapy data are available. Provera has some activity.

E. Hepatoma

There is preliminary evidence of adriamycin activity but the U. S. data are minimal.

Without entering into a detailed account of the state of knowledge in chemotherapy, the priorities of study in the hematologic malignancies are also included in Table 1.

Evaluation of the suggested priorities of study for use in the allocation of Phase II testing resources with the 18 solid tumors produces 3 groupings (Table 2) according to the requirements of drug evaluation.

Only 2 of the 4 tumors in Group I fall into the category requiring high priority

Table 2. Comparison of individual tumors and the priorities for Phase II clinical evaluation.

Tumor Group	Priority Areas for Drug Evaluation		
	New Investigational Drugs	Standard Drugs Not Previous Tested	Combinations
I	Lung Colon	Pancreas	Breast
II		Stomach Prostate Brain	Ovary Cervix Head & Neck Testicular Sarcomas
III	Melanoma	Bladder Kidney Esophagus Endometrial Hepatoma	
Hematologic malignancies	AML	ALL	CML CLL Myeloma Hodgkin's disease Non-Hodkin's lymphomas

for evaluating new investigational agents. However, these two are lung and large bowel cancer, which have the highest incidence and mortality in the United States.

Nine of the solid tumors are so lacking in data for the standard agents that this facet of drug evaluation should receive the highest priority. In these tumors, it is more reasonable to test a drug having known cell kill potential against some type of cancer rather than studying a totally unproven drug.

Six solid tumors are responsive enough to standard chemotherapeutic agents that combinations should receive the greatest impetus with available clinical resources.

Table 2 also includes the approaches to drug testing that merit the greatest emphasis in the various hematologic malignancies.

The Cancer Therapy Evaluation Program, working closely with the Associate Director for Medical Oncology and his staff, is attempting to expand the clinical resources available to the DCT to implement as much as possible of the above program and to integrate on-going studies with the clinical resources supported by other divisions of the NCI (Table 3).

As part of the attempt to effectively coordinate the disparate clinical resources supported in 3 of the 4 NCI division, the CTEP staff travels extensively, participating in many extramural treatment groups, and sponsors a variety of meetings that attempt to consolidate the significant volume of information which is continually developing.

The CTEP staff attends nearly all meetings of the cooperative clinical groups sponsored by Clinical Investigations Branch of the Division of Cancer Research Resources and Centers. The CTEP provides liaison from the Investigational Drug Branch concerning DCT investigational drugs being used by the groups and furnishes each group with up-to-date knowledge of progress in other NCI supported treatment resources.

The Associate Director for Cancer Therapy Evaluation actively participates in the following disease-oriented task forces and working cadres:

1. Breast Cancer Task Force - Treatment Committee Member
2. National Prostatic Cancer Project - Member of working cadre and Treatment Committee.
3. National Large Bowel Cancer Project - Member of Advisory Committee
4. National Pancreatic Cancer Project (proposed) - Member of working cadre.
5. Lung Cancer Working Party - Member Chemotherapy Committee.

The CTEP continues to undertake major responsibility for the clinical aspect of international liaison with the DCT. The Associate Director continues as co-project officer for the contract supported liaison offices in Brussels and Tokyo. The Brussels liaison office, as one of its functions, coordinates the E.O.R.T.C. cooperative group studies with on-going work at the NCI. The Associate Director has been appointed a member of the newly formed Protocol Review Commit-

Table 3. Disease-oriented treatments efforts in various divisions of the National Cancer Institute (Revised 11/6/73).†

Tumor Types	Intramural Studies				Extramural Studies			
	Surgery (DCBD)	Radiation (DCBD)	Immunotherapy (DCBD)	Chemotherapy (DCT)	DCT Contracts	DCBD Contracts (Immunotherapy)	Collaborative Program Cooperative Groups (DCRRC)	Task Forces and Cadres (DCRRC & DCBD)
Lung	-	+	-	++	++	+	++	-
Breast	++	-	-	++	++	⊕	++	++*
Large Bowel	-	-	-	-	++	⊕	++	++
Pancreas	-	-	-	-	++	-	+	(++)
Stomach	-	-	-	-	++	-	+	-
Ovary	-	-	-	+	+	-	++	-
Prostate	-	-	-	⊕	⊕	-	+	++
Bladder	-	-	-	-	-	-	+	++
Cervix	+	-	-	-	-	-	+	-
Head and Neck	++	+	-	-	-	-	+	(++)
Testicular	-	-	-	-	++	-	+	-
Brain	-	-	-	++	++	-	+	-
Melanoma	+	-	++	+	+	++	++	-
Sarcoma	+	+	-	+	⊕	+	++	-
Lymphoma	-	++	-	++	-	+	++	-
AML	-	-	+	++	-	+	++	-
ALL	-	-	+	++	-	+	++	-
Pediatric Solid Tumors	-	+	-	++	-	-	++	-
Kidney	-	-	-	-	-	-	+	-
Esophagus	-	-	-	-	-	-	+	-
Chronic Leukemia	-	-	-	+	-	-	+	-
Myeloma	-	-	-	+	-	-	++	-

† Abbreviations: DCBD = Division of Cancer Biology & Diagnosis; DCT = Division of Cancer Treatment; DCRRC = Division of Cancer Research Resources & Centers

++ = Major effort

+ = Some studies being performed

⊕ or (++) - In planning stage

* = Breast Cancer Task Force in DCBD

tee of the E. O. R. T. C., which will evaluate all new protocols devised by their groups. The Tokyo liaison office, as one of its functions, is seeking a greater coordination of Japanese clinical studies with those at NCI. One of the results of this collaboration has been the formation of Phase I working groups in Japan by Professor Kioji Kimura (Vice Director, National Cancer Center, Tokyo). These groups have been formed to study drugs developed in the DCT program.

The Associate Director continues his active role in the Joint U. S. - U. S. S. R. agreement on cancer chemotherapy signed in June 1972. A third trip to the Soviet Union was made in March 1974 to further implement the drug exchange, develop a joint study on lung cancer, and work on a joint publication in the field of drug development.

HONORS AND AWARDS

1. Carter, S. K.: Appointed as co-editor of new journal, Cancer Treatment Reviews.

2. Carter, S. K.: Appointed to editoral board, Seminars in Oncology.

3. Carter, S. K.: Appointed to editoral board of new journal, Journal of Medical and Pediatric Oncology.

PUBLICATIONS

1. Carter, S. K.: The choice of therapy in the treatment of malignancy. Med. Lett. Drugs Ther. 15:9-16, 1973.

2. Carter, S. K.: New drugs on the horizon in gastrointestinal cancer. In: Estratto da Corso Superiore sul Trattamento dei Tumori del Sistema Digestio, Milano, 22-26 Maggio 1972. Milano, Casa Editrice Ambrosiana, 1973, pp. 317-327.

3. Carter, S. K.: The status of chemotherapeutic approach to gastrointestinal cancer at the National Cancer Institute. In: Estratto da Corso Superiore sul Trattamento dei Tumori del Sistema Digestivo, Milano, 22-26 Maggio 1972. Milano, Casa Editrice Ambrosiana, 1973, pp. 99-117.

4. Carter, S. K.: An overview of the status of the nitrosoureas in other tumors Cancer Chemother. Rep. (Part 3) 4:35-46, 1973.

5. Carter, S. K.: Some thoughts on surgical adjuvant studies in lung cancer. Cancer Chemother. Rep. (Part 3) 4:109-117, 1973.

6. Carter, S. K.: Planning combined therapy-The interaction of experimental and clinical studies. Cancer Chemother. Rep. (Part 3) 4:3-11, 1974.

7. Carter, S. K.: Current therapy approaches of the Division of Cancer Treatment with emphasis on pancreatic carcinoma. J. Surg. Oncology 6:9-17, 1974.

8. Carter, S. K.: Useful agents in lymphomas and solid tumors. Consultant

13:45-48, 1973.

9. Carter, S. K: What's new in cancer therapy? Consultant 13:135-137, 1973.

10. Carter, S. K.: Introduction to methodology of clinical trials in the varieties of acute leukemia; defining the numerator and denominator in leukemic trials. Resent Results in Cancer Res. 43:107-109, 1973.

11. Carter, S. K. and Livingston, R. B.: Single agent therapy for Hodgkin's disease. Arch. Intern. Med. 131:377-388, 1973.

12. Carter, S. K. and Soper, W. T.: Integration of chemotherapy into a combined modality treatment of solid tumors. I. The overall strategy. Cancer Treatment Reviews 1:1-13, 1974.

13. Broder, L. E. and Carter, S. K.: Pancreatic islet cell carcinoma: I. Clinical features of 52 patients. Ann. Intern. Med. 79:101-107, 1973.

14. Broder, L. E. and Carter, S. K.: Pancreatic islet cell carcinoma: II. Results of therapy with streptozotocin in 52 patients. Ann. Intern. Med. 79: 108-118, 1973.

15. Goldin, A. and Carter, S. K.: Screening and evaluation of antitumor agents In: Cancer Medicine (J. F. Holland and E. Frei, Eds.), Philadelphia, Lea and Febiger, 1973, pp. 605-628.

16. Blum, R. H., Livingston, R. B., and Carter, S. K.: Hexamethylmelamine - A new drug with activity in solid tumors. Europ. J. Cancer 9:195-202, 1973.

17. Blum, R. H. and Carter, S. K: Pulmonary complications of cancer chemotherapy. New Eng. J. Med. 288:266, 1973.

18. Blum, R. H., Carter, S. K., and Agre, K.: A clinical review of bleomycin A new antineoplatic agent. Cancer 31:903-214, 1973.

19. Blum, R. H. and Carter, S. K.: Review of adriamycin - A new anticancer drug with significant clinical activity. Ann. Intern. Med. 80:249-259, 1974.

20. Goldsmith, M. A. and Carter, S. K.: 4'Demethyl-epipodophyllotoxin-β-D-thenylidene glucoside (VM-26) - A brief review. Europ. J. Cancer 9:477-482, 1973.

21. Goldsmith, M. A. and Carter, S. K.: Combination chemotherapy of advanced Hodgkin's disease: A review. Cancer 33:1-8, 1974.

22. Wasserman, T. H. and Stuard, I. D.: Widespread metastases of malignant fibrous histiocytoma. Cancer 33:141, 1974.

PRESENTATIONS

1. Carter, S. K.: The Division of Cancer Treatment's Approach to the Testing of New Anticancer Drugs -- example cis-platinum diamminedichloride.

Second International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy. Oxford, England, April 18, 1973.

2. Carter, S. K.: Clinical Trial Approaches with New Drugs and their Integration into Disease-Oriented Combined Modality Attacks. E. O. R. T. C. Symposium on Clinical Pharmacology, Brussels, Belgium, May 7-11, 1973.

3. Carter, S. K.: Specific Design Considerations in Gastrointestinal Cancer. E. O. R. T. C. Symposium on Clinical Pharmacology, Brussels, Belgium, May 7-11, 1973.

4. Carter, S. K.: Drug Aspects in the Design of Surgical Adjuvant Studies for Breast Cancer. NCI Breast Cancer Treatment Meeting, Holiday Inn, Bethesda, May 17-18, 1973.

5. Carter, S. K.: Phase II Studies in Breast Cancer. NCI Breast Cancer Treatment Meeting, Holiday Inn, Bethesda, May 17-18, 1973.

6. Carter, S. K.: Where are We Today in Cancer Treatment? Tokyo University Hospital, May 23, 1973.

7. Carter, S. K.: The Strategy for a Combined Modality Approach to Cancer Therapy. Dedication Ceremony Address, New Chemotherapy Center, Japanese Institute for Cancer Research, Tokyo, May 24, 1973.

8. Carter, S. K.: The Combined Modality Approach to Cancer Therapy, Conference on Chemotherapy, National Cancer Center, Tokyo, May 25, 1973.

9. Carter, S. K.: The Problems of Correlating Animal and Clinical Data. DCT Advisory Committee Meeting, Bethesda, June 11, 1973.

10. Carter, S. K.: Medical Perspectives in Gastrointestinal Cancer Therapy. ACP Course on Medical Oncology and Chemotherapy, Los Angeles, June 16, 1973.

11. Carter, S. K.: A Clinical Approach to Analog Development with Emphasis on the Nucleoside Area. DCT Nucleoside Review Meeting, Bethesda, June 21, 1973.

12. Carter, S. K.: Some Thoughts on Experimental Models and Their Clinical Correlations with Emphasis on Combination Approaches. 2nd Annual Meeting of the International Society for Experimental Hematology, Paris, France, June 28, 1973.

13. Carter, S. K.: From Animal to Man in Toxicology. Annual Meeting of E. O. R. T. C., Paris, France, June 29, 1973.

14. Carter, S. K.: New Drugs Available for Choriocarcinoma. E. O. R. T. C. Choriocarcinoma Group Meeting, Villejuif, France, June 30, 1973.

15. Carter, S. K.: Design Considerations in Breast Cancer. NCI Medical Breast Cancer Seminar, Bethesda, August 27, 1973.

16. Carter, S. K.: New Anticancer Drugs Sponsored for Clinical Trials by the DCT. International Congress on Chemotherapy, Athens, Greece, September 9, 1973.

17. Carter, S. K.: Antibiotic Chemotherapeutic Agents. Medical Oncology Course of M. D. Anderson Hospital, Houston, September 28, 1973.

18. Carter, S. K.: The Chemotherapy of Common Solid Tumors. Medical Oncology Course of M. D. Anderson Hospital, Houston, September 28, 1973.

19. Carter, S. K.: Considerations in the Use of Chemotherapy in Surgical Adjuvant Studies in Breast Cancer. National Surgical Adjuvant Breast Project Group Meeting, Bethesda, October 10, 1973.

20. Carter, S. K.: Design Considerations in Clinical Trials. State University of Buffalo Biometry Seminar, Buffalo, November 2, 1973.

21. Carter, S. K.: Clinical Studies in Combined Modality. Fourth Joint Working Conference of DCT, Hunt Valley Inn, November 19, 1973.

22. Carter, S. K.: NCI Treatment Approaches in Pancreatic Cancer. National Cancer Advisory Board Meeting, Bethesda, November 26, 1973.

23. Carter, S. K.: Adriamycin in the Treatment of Solid Tumors. Adriamycin Meeting, Paris, France, December 19, 1973.

24. Carter, S. K.: How to Treat Advanced Breast Cancer. University of California, Cancer Research Institute Seminar, January 25, 1974.

25. Carter, S. K: The Current Approach of the DCT to Large Bowel Cancer. First Annual Workshop, Large Bowel Cancer Project, Houston, January 31, 1974.

26. Carter, S. K.: The DCT and Cooperative Groups Clinical Program in Breast Cancer. Annual Program Review Conference of the Breast Cancer Task Force, Denver, February 4-6, 1974.

27. Carter, S. K.: An Overview of Clinical Status of CCNU, DTIC, and Hexamethylmelamine. U. S. -U. S. S. R. Chemotherapy Agreement Review, Institute for Experimental and Clinical Oncology, Moscow, March 13-14, 1974.

28. Carter, S. K.: An Overview of Clinical Data on the Nitrosoureas. U. S. -U. S. S. R. Chemotherapy Agreement Review, Institute for Experimental and Clinical Oncology, Moscow, March 13-14, 1974.

29. Carter, S. K.: The Clinical Status of Adriamycin. U. S. -U. S. S. R. Chemotherapy Agreement Review, Institute for Experimental and Clinical

Oncology, Moscow, March 13-14, 1974.

30. Carter, S. K.: Status of Asaley Trials in the U.S. U.S.-U.S.S.R. Chemotherapy Agreement Review, Intitute for Experimental and Clinical Oncology, Moscow, March 13-14, 1974.

31. Carter, S. K.: Surgical Adjuvant Approaches Sponsored by the NCI. American Collegeof Surgeons Meeting, Houston, March 26, 1973.

32. Comis, R. L.: The Chemotherapy of Malignant Melanoma: A Review. Symposium on New Trends in Experimental and Clinical Research on Melanoma. WHO International Reference Center for Diagnosis and Treatment of Melanoma, Oslo, Norway, June 15, 1973.

33. Wasserman, T. H.: Clinical Review of CCNU and MeCCNU. Western Cancer Study Group Meeting,San Francisco, September 10, 1973.

34. Wasserman, T. H.: Clinical Overview and Comparisons of the Nitrosoureas. New Drug Liaison Meeting, Bethesda, December 10, 1973.

35. Wasserman, T. H.: Progress in Cancer Chemotherapy. Florida Osteopathic Seminar, Miami, December 15, 1973.

LITERATURE RESEARCH SECTION

This Section is now located within the Office of the Associate Director for Cancer Therapy Evaluation and provides literature research and services supporting the Cancer Treatment Program. Data from the fields of chemotherapy, radiotherapy, surgery, immunotherapy, chemistry, and biochemistry is used in Decision Network review, as back-up material meeting FDA requirements for IND filing, and as background for toxicological and clinical evaluations.

Approximately 150 requests for literature searches were filled during the year by selective bibliographies, computer print-outs, and copies of articles. Bibliographies on clinical diseases included natural history of stomach and pancreatic cancer, chemotherapy of head and neck tumors, eye tumors, and bladder cancer, and radiotherapy and surgery of stomach cancer. Bibliographies on daunomycin and adriamycin were updated and searches were performed for such agents as streptozotocin, 5-azacytidine, dibromodulcitol, isophosphamide, cis-diamminedichloroplatinum, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide, and the Russian drugs fluorodopan, ftorafur, and asaley. A continuing project is a monthly search for adverse effects of antitumor agents in clinical use.

Computer services of the National Library of Medicine and the Division of Computer Research and Technology aid literature searching and facilitate answerin of requests. An agreement with NLM provides on-line access to the MEDLINE data base. An expanded data base and improved searching capabilities this yea have greatly increased the usefulness of the system. The Section terminal is available for use by all NCI personnel. DCRT annotated bibliographies provide an alerting service for new and potential antineoplastic agents. The printouts, from a biweekly search of CBAC tapes from Chemical Abstracts Service, are circulated to staff members.

This year the Section compiled and distributed the Cancer Treatment Program Statistics. Statistics are cumulated quarterly for the number and types of materials entering the DR&D Biological Processing System, tests performed, number and types of confirmed actives in the primary screen, compounds passing each Decision Network step, and drugs for which INDs are filed.

The Section also maintains the Cancer Treatment Program Library. Over 1600 journals were circulated this year and approximately $14,000 was spent on books, journal subscriptions, and binding. Plans are underway to furnish a second library in the Blair Building.

Contracts for preparing of Cancer Chemotherapy Abstracts, have been monitored by the Section since 1965. This year, reflecting the broadened DCT Program, journal coverage was expanded to include surgery, radiotherapy, and immunotherapy of cancer. The first issue of Cancer Therapy Abstracts, replacing CCA, will be published in the summer of 1974.

In addition to the printed journal, the Section receives hard copy of all articles cited and computer tapes of abstracts and indexes. A pilot project, sponsored by the Office of Program Planning and Analysis, to reformat CCA tapes to produce a magnetic tape for incorporation in the NLM system is nearing completion. It is planned that CCA and CTA tapes will form a separate data base that can be searched on-line through the MEDLINE network.

COMBINED MODALITY BRANCH

Historically, the Division of Cancer Treatment (DCT) has evolved from the chemotherapy program, where for many years the major thrust was directed to the development of new anticancer agents. However, the National Cancer Act of 1971 has vested in the DCT the broader responsibility of leading the national effort to control cancer through the therapeutic modalities of surgery radiotherapy, chemotherapy, and immunotherapy. Accordingly, the Combined Modality Branch is attempting to develop effective treatment approaches through the optimum use of all modalities and clinical resources.

Clinical experience suggests that each type of neoplastic disease presents different therapeutic requirements that should be met by an individualized treatment strategy. Thus, the first feature of the overall approach to combined modality therapy is that it must be disease-oriented.

Another critical concept is that the best chance for cure lies in the primary treatment (i. e., surgery and radiotherapy) of solid tumors without disseminated disease. However, it is becoming increasingly clear that disseminated microscopic disease foci, undetectable by present techniques, already exist a the time of primary treatment. Thus, the curative potential of the local modalities of surgery and radiotherapy is limited by their inability to reach and eradicate the last tumor cell when detection techniques indicate that the disease is still localized. On the other hand, chemotherapy and, possibly immunotherapy can destroy neoplastic cells anywhere in the body and offer the potential for eradicating the metastatic foci of early disease. Unfortunately, chemotherapy is usually relegated to secondary or tertiary treatment after the local modalities fail and the disease is obviously disseminated. Such late therapy is rarely curative in any disease.

It has been found that the systemic modalities exert their maximum effect when the tumor cell population is small, i. e., very early in the disease or immediately after surgical removal of the primary tumor mass. Thus, the local and systemic modalities are therapeutically complementary. Accordingly, the second feature of the combined modality approach is that it must integrate all modalities into single strategies for the primary treatment of specific disease.

Implementation of a disease-oriented combined modality approach to primary treatment is an essential and timely undertaking requiring the following action by the DCT:

1. Establishment of priorities among tumors based on disease incidence, mortality, and the probability of success.

2. Development of therapeutic strategies for the highest priority tumor types according to disease type and extent.

3. Definition of existing and new resources required to carry out the program.

4. Integration and coordination of treatment resources to achieve

the established goals.

In setting the priorities for combined modality studies, each tumor must be considered within the following criteria: (a) incidence and severity of the disease at the national level, (b) the potential of surgery and radiotherapy in disease control, (c) the cell kill potential of chemotherapy and immunotherapy, and (d) the existence of intermodality coordination channels.

Table 1 presents a preliminary ranking of 16 solid tumors according to their potential responsiveness to combined therapy on the basis of the above criteria. At one extreme, breast cancer is the leading cause of cancer deaths in women, most patients are amenable to surgery of curative intent at the time of initial diagnosis, and chemotherapy offers good cell kill potential. At the opposite extreme, esophageal cancer is relatively uncommon, surgery and/or X-ray afford no curative potential, and there is no effective chemotherapy at present. The other 14 tumors fall between these two extremes.

A critical point in developing an overall treatment strategy is the fact that surgery and radiotherapy have the highest curative potential in the early stage of any solid tumor. Consequently, their role in the new approach must be emphasized. Obviously, single modality development and/or refinement must precede their integration into a combined modality approach.

The proposed therapeutic approach of the DCT for increasing cure rates in solid tumors involves the following sequential strategy:

> Independent testing of new procedures of surgery, radiotherapy, chemotherapy and, possibly immunotherapy, in cancer patients as early as ethically permissible.
>
> Development of optimum single modality regimens for primary treatment of disseminated disease.
>
> Integration of optimum single modality regimens into a combined modality approach to primary treatment of local and regional disease.

In this scheme, new modality-specific procedures would be developed and tested in advanced disease and those showing positive results would be moved into the primary therapy of disseminated disease. The optimum regimen evolved in this situation would then be integrated into a combined modality approach for primary treatment in local and regional disease.

The definition, integration, and coordination of clinical resources essential for the combined modality approach is complicated by the multitude of intramural and extramural efforts now underway for identical disease and the lack of coordination among them. For example, in the area of breast cancer therapy intramural and contract programs are progressing in the DCT, while concomitant studies are being conducted in 6 cooperative groups supported by the Division of Cancer Resources and Centers. A plurality of efforts is not necessarily wrong because no single group has a monopoly on good ideas regarding therapy. However, it is unfortunate that this approach produces overlapping efforts, an absence of standard protocols for identical disease situations, dispersion of resources, a lack of uniform definitions and data reporting techniques, poor coordination, and inadequate exchange of information--all leading to decrease operational efficiency and reduced cost-effectiveness.

Broad Requirements for Development of a Combined Modality Approach (Modality Integration)

Regarding the broad requirements for development of a combined modality approach, each modality employing chemotherapy can be handled in 4 interrelat ways: preclinical or clinical, and modality-oriented or disease-oriented. Cu rently, the Drug Development Program begins with a preclinical base that is totally drug-oriented. It then moves into the clinical area where testing begi with drug-oriented Phase I trials but progresses toward disease-oriented Pha II and III studies. It would be useful to approach each combined modality program in the same manner, and accomplish it in a way that both grows from an integrates with the current drug development program.

The following is an example of the way in which this approach might proceed i combining radiotherapy and chemotherapy, an area to be given increased DCT attention during the next year:

A. Screening. All known active antitumor agents and those passing Decision Network 2 should be tested for potential synergism with radiotherapy. Many in vitro and in vivo systems have been used for such testing in the past, but not on a large scale. Such tests could identify the active drugs that should receive highest priority for clinical trials with radiotherapy. Development of a relatively inexpensive in vitro system would broaden the potential for screening of analogs of active agents in this area.

B. Toxicology. Efforts should be made to develop toxicology models for evaluating and predicting toxic effects that might occur when a specific drug is combined with radiotherapy. These models should be organ-specific (i. e., bowel, kidney, liver, etc.) as well as specific for the animal. Ideally, rodents would be less expensive but dogs and/or monkeys may be required. If a drug passes DN 2 and shows a high potential for synergism with radiotherapy, some radiotherapy combination studies might be included in the basic toxicologic evaluation of the drug.

C. Schedule Dependency. Experimental systems should be found or further developed to evaluate the optimum schedule for a given drug used with radiotherapy. The system should also be capable of identifying optimum time-dose relationships so that clinical testing can be directed to either drug→X-ray, X-ray→drug, or concomitant use of the two.

D. Clinical Testing.

1. Phase I trials would remain modality-oriented and establish the maximum tolerated dose (MTD) of each drug combined with radiotherapy to fields involving marrow production. Although the curative potential might be small or non-existent, trials would be conducted only in patients with tumors for which radiotherapy is ethi-

cally feasible. Using the MTD established for the drug alone, these studies would hopefully be small ones employing few patients and giving rapid answers.

2. Phase II studies flowing rapidly from Phase I results would be disease-oriented in solid tumors using individual approaches for each tumor. Concise, controlled study designs should be evolved to determine whether the drug added to radiotherapy enhances the therapeutic response. Essential in this design would be a detailed review of past results with radiotherapy alone in the specific disease, including both response and relapse rates. If several such trials using both a concomitant and a historical control indicate the validity of the latter, it could be more heavily used to conserve patient resources.

3. Phase III disease-oriented trials hopefully would deemphasize the old, almost total, reliance on massive studies to prove whether drug + X-ray is better than X-ray alone down to the 10% level. This difference should be set higher in future studies but the major emphasis should be placed on determining an optimum combination for earlier use in the disease-oriented therapy that will increase the curative potential of the primary therapeutic approach.

4. Exploration of new approaches should emphasize the prophylactic use of radiotherapy in areas that, for a given disease, present a high risk of developing a metastatic focus or recurrence. The classic example of this approach is the radiation therapy of the CNS in childhood acute leukemia. In lung cancer, expecially oat cell lesions, thought is being given to radiotherapy for areas such as the brain, liver, and adrenals. Much of this radiotherapy would be concomitant with drug treatment and exploration of possible organ-specific problems that could arise with various drugs should receive high priority consideration.

One of the major thrusts of the Combined Modality Branch has been in the area of gastrointestinal cancer through the formation of a contract supported Gastrointestinal Tumor Study Group which has written protocols for pancreatic cancer and gastric cancer and will now begin consideration of protocols for large bowel cancer. The group has recently elected two co-chairmen who will scientifically lead the group, while administrative support is given by the CancerTherapy Evaluation Program. These chairmen are Dr. Charles Moertel and Dr. Philip Schein.

In order to compile a needed data base in gastrointestinal cancer, Combined Modality Branch has reviewed the current protocols in gastrointestinal cancer. The tables in Appendix A summarize the major current protocols for the three most common gastrointestinal malignancies, i.e., colorectal, pancreatic, and gastric. These studies include surgical adjuvant therapy, regimens for regional disease, and those for advanced disease. The following table gives a breakdown for each study category being pursued in each major malignancy.

Malignancy	Total No. of Studies	Study category Surgical Adjuvant	Regional	Advanced
Colorectal	19	8	4	7
Pancreas	12	1	3	8
Stomach	16	6	2	8
Totals	47	15	9	23

It can be seen that, in general, the character of the studies parallels the biology of the disease. The greatest proportion of surgical adjuvant studies is devoted to colorectal cancer where the number of patients receiving potential curative resection is greatest. In contrast, there is only one surgical adjuvant study in pancreatic cancer where the curative resection capability is minimal.

Although the number of gastric surgical adjuvant studies is listed as 6 of the total 16 studies, only 2 of these are being carried out in the U.S. Three major studies are in progress in Japan and one in Europe. It is impressive to note that about one-third of the studies are testing the value of bringing chemo therapy and/or radiation into the coordinated attack in patients with early disease. All of these studies are randomized and most are controlled and reasonably well designed.

Of critical importance is the interaction between the various aspects of NCI supported research in the treatment of specific gastrointestinal tumors. This is explored for large bowel cancer in Appendix B.

APPENDIX A. MAJOR CURRENT PROTOCOLS IN GASTROINTESTINAL CANCER

Table 1. Carcinoma of the stomach.

Modality	Patient Profile	Compared Groups	Investigators
Chemotherapy	Surgical Adjuvant (Curative Resection)	No Treatment vs 5-FU 400 mg/m^2/d IV x 5 q 5 wks + BCNU 40 mg/m^2/d IV x 5 q 10 wks (Treatment to relapse or 2 yrs)	Dr. H. D. Douglas, Jr. Roswell Park Memorial Institute Gastrointestinal Tumor Study Group Protocol
Chemotherapy	Advanced Cancer (Measurable Disease)	MTX 80 mg/m^2 IV q 2 wks vs 5-FU 325 mg/m^2 IV d 1-5 & 36-40 + Mito-C 2 mg/m^2 IV d 1-5 & 36-40 vs MeCCNU 175 mg/m^2 single dose PO + 5-FU 325 mg/m^2 IV d 1-5 & 36-40 (Crossover on Progression)	Dr. C.G. Moertel Mayo Clinic Eastern Cooperative Oncology Group Protocol
Chemotherapy	Advanced Disease	CTX 1200 mg/m^2 IV followed by MeCCNU 200 mg/m^2 PO vs CTX 1200 mg/m^2 IV followed by MeCCNU 175 mg/m^2 PO + 5-FU 300 mg/m^2/d x 5 vs MeCCNU 200 mg/m^2 PO vs MeCCNU 175 mg/m^2 PO + 5-FU 300 mg/m^2/d x 5	Dr. C.G. Moertel Mayo Clinic Eastern Cooperative Oncology Group Protocol

Chemotherapy	Advanced Cancer (Non-measurable Disease)	5-FU 450 mg/m^2/d IV x 5 q 5 wks vs 5-FU 450 mg/m^2/d IV x 5 + Testolactone 50 mg t. i. d. vs 5-FU 325 mg/m^2 IV d 1-5 & 36-40 + MeCCNU 175 mg/m^2 PO single dose vs 5-FU + MeCCNU + Testolactone Progression MTX 80 mg/m^2 IV q 2 wks x 10, then q 3 wks ± Testolactone	Dr. C. G. Moertel Mayo Clinic Eastern Cooperative Oncology Group Protocol
Chemotherapy	Surgical adjuvant (Curative, palliative or unresectable)	No treatment vs 5-FU 9 mg/kg/d IV x 5 q 7 wks + MeCCNU 120 mg/m^2 single dose q 7 wks	Dr. G. A. Higgins, Jr. VA Hospital, Wash. D. C. VA Surgical Adjuvant Cancer Chemorx. Study Group Protocol
Chemotherapy	Inoperable, residual, recurrent or metastatic	MeCCNU 200 mg/m^2 PO q 6 wks vs MeCCNU 120 mg/m^2 PO q 6 wks + CTX 600 mg/m^2 IV q 6 wks Progression MTX 30 mg/m^2 IM 2x wkly	Dr. G. Bonnadonna NCI, Milan, Italy
Chemotherapy	Inoperable or metastasis post operative	CCNU 100 mg/m^2 PO q 6 wks vs CCNU 75 mg/m^2 PO q 6 wks + 5-FU 9 mg/kg IV d 1-4 & 22-24 vs 5-FU 12 mg/kg d 1-4 & 22-24 vs CCNU 75 mg/m^2 PO q 6 wks + Ftorafur 1. 5 gm IV d 1-6 x 3 wks vs	Prof. N. N. Blokhin Institute of Experimental and Clinical Oncology Moscow, USSR

Chemotherapy	Progressive disease unresponsive to standard therapy	MTX 60 mg/m^2/wk IV x 12 vs MTX 15 mg/m^2 /6 hrs PO x 4, wkly x 12 vs MTX 125 mg/m^2/6hrs PO x 4 wkly x 12 + Leucovorin 5 mg/6hrs PO x 6, begining 36 hrs after first dose of MTX	Dr. C. Vogel Grady Memorial Hospital Atlanta, Georgia Southeastern Cancer Study Group Protocol
Chemotherapy	Inoperable, recurrent, palliative resection & metastatic disease	Neocarzinostatin 3-6 mg/d IV x 20	Dr. Y. Aneba National Sendai Hospital, Japan Gastric Cancer Cooperative Research Group
Chemotherapy	Gastric Resection	No treatment vs 5-FU 5 mg/kg IV d1-5, then 10 mg/kg IV QOD (total 150 mg/kg) + Mito-C 8 mg IP + 0.2 mg/kg IV (during surgery) + CTX 4 mg/kg IV d2-15	Dr. T. Kimura First National Hospital Tokyo, Japan Gastric Cancer Cooperative Research Group
Chemotherapy	Curative Resection	No treatment vs Mito-C 4mg IP(during surgery) + 0.12 mg/kg IV + 5-FU 10 mg/kg IV 2x wk x 3 vs Mito-C 0.2 mg/kg IV during surgery + 5-FU 10 mg/kg QOD, d4-20	Dr. T. Kimura First National Hospital Tokyo, Japan Gastric Cancer Cooperative Research Group

Chemotherapy	Curative Resection	No treatment vs Mito-C 0.08mg/kg IV 2x wk x 5 vs Mito-C 0.04 mg/kg IV 2x wk (20 mg/50 kg total) + 5-FU 5 mg/kg IV 2x wk (2500 mg/50 kg total) + Ara C 0.4 mg/kg IV 2x wk (200 mg/50 kg total)	Dr. H. Imanaga Aichi Cancer Center Nagoya, Japan Cooperative University Group of Surgical Adjuvant Chemotherapy
Chemotherapy + Radiation Therapy	Curative or palliative resection	Radiation therapy(5600r in 6wks) vs Radiation therapy(5600r in 6wks) + 5-FU 375 $mg/m^2/d$ x 4 ± 5-FU 500 mg/m^2/wk IV (post radiation therapy)	Dr. A. Gerard St. Pierre University Hospital Brussels, Belgium EORTC Cooperative Group Protocol
Chemotherapy + Radiation Therapy	Locally unresectable	5-FU 600 mg/m^2/wk IV vs Radiation therapy (4000r) + 5-FU 600 mg/m^2 IV d1-3 + 5-FU 600 mg/m^2/wk IV following radiation therapy	Dr. D. J. Klassen University of Ottawa Canada Eastern Cooperative Oncology Group Protocol
Chemotherapy + Radiation Therapy	Locally advanced disease (resected & nonresected)	5-FU 500$mg/m^2/d$ IV x 5 q 5wks vs 5-FU 400$mg/m^2/d$ IV x 5 q5wks + BCNU 40 $mg/m^2/d$ x 5 IV q 10 wks vs Radiation therapy 5000r split course + 5-FU 500 mg/m^2 d1-3 of each course RT followed by 5-FU 400$mg/m^2/d$ IV x 5 q 5wks + BCNU 40 $mg/m^2/d$ x 5 IV q 10 wks after radiation therapy	Dr. R. C. DeConti Yale University School of Medicine Gastrointestinal Tumor Study Goup Protocol

Immunotherapy	Advanced disease (failed prior therapy)	MER-BCG 0.4 mg/wk x 8 in each of 5 sites, then q 4 wks (if no progression) vs Q 4 wk schedule x 2 then q 4 wks (if no progression)	Dr. C.G. Moertel Mayo Clinic

Table 2. Carcinoma of the pancreas.

Modality	Patient Profile	Compared Groups	Investigators
Chemotherapy	Advanced measurable disease (untreated)	Melphalan 9mg/m^2/dx4 PO q 5 wks vs Streptozotocin 500 mg/m^2 IV d1-5 & 35-39 + 5-FU 350 mg/m^2 IV d1-5 & 35-39 + MeCCNU 150 mg/m^2 PO d1 vs Streptozotocin 500 mg/m^2/d x5 IV q 5 wks + 5-FU 400 mg/m^2/d IV x 5 q 5 wks	Dr. C.G. Moertel Mayo Clinic Eastern Cooperative Oncology Group Protocol
	Previously treated with 5-FU or nitrosourea	Melphalan 9 mg/m^2/d PO x 4 q 5 wks	
	Previously treated with alkylating agent	Streptozotocin 500 mg/m^2/d IV x 5 q 5 wks + 5-FU 400 mg/m^2/d IV x 5 q 5 wks vs Streptozotocin 500 mg/m^2 IV d1-5 & 35-39 + 5-FU 350 mg/m^2 IV d1-5 & 35-39 + MeCCNU 150 mg/m^2 PO d1	

Chemotherapy	Advanced nonmeasurable disease (untreated)	5-FU 450 mg/m^2/dx5 IV q5wks vs 5-FU 450 mg/m^2/dx5 IV q5wks + Spironolactone 50 mg tid vs 5-FU 400 mg/m^2/dx5 IV q 5wks + Streptozotocin 500 mg/m^2/d x 5 IV q 5 wks vs 5-FU 450 mg/m^2/dx5 IV q 5wks + Streptozotocin 500 mg/m^2/d x 5 IV q 5 wks + Spironolactone 50 mg tid	Dr. C.G. Moertel Mayo Clinic Eastern Cooperative Oncology Group Protocol
Chemotherapy	Advanced disease (untreated)	MeCCNU 250 mg/m^2 PO vs CTX 1000 mg/m^2 IV d1&21 + Streptozotocin 500 mg/m^2/dx5 IV vs Streptozotocin 500 mg/m^2/dx5 IV + 5-FU 400 mg/m^2/d x 5 IV	Dr. C.G. Moertel Mayo Clinic Eastern Cooperative Oncology Group Protocol
	Previously treated with 5-FU	Streptozotocin 500 mg/m^2/dx5 IV + CTX 1000 mg/m^2 IV d 1&21 vs MeCCNU 250 mg/m^2 PO	Dr. C.G. Moertel Mayo Clinic Eastern Cooperative Oncology Group Protocol
	Other than 5-FU	Streptozotocin 500 mg/m^2/dx5 IV + 5-FU 400 mg/m^2/d x 5 IV vs MeCCNU 250 mg/m^2 PO	Dr. C.G. Moertel Mayo Clinic Eastern Cooperative Oncology Group Protocol
Chemotherapy	Advanced disease	5-FU 12.5 mg/kg IV d1-5, then wkly vs 5-FU 12.5 mg/kg IV d1-3, 17, 31, &45 + Tubercidin 1500gamma/kg IV d1 + Streptozotocin 12.5 mg/kg IV d10, 24, &38	Dr. W.S. Fletcher University of Oregon Medical School Central Oncology Group Protocol

Chemotherapy	Locally nonresectable	No treatment vs CCNU 70 mg/m^2 PO single dose q 6 wks + 5-FU 9 mg/kg/d x 5 IV q 6wks	Dr. G. A. Higgins, Jr. VA Hospital, Wash. D. C. VA Surgical Adjuvant Cancer Chemorx. Study Group Protccol
Chemotherapy	Unresponsive to standard therapy	MTX 60 mg/m^2/wk IV x 12 vs MTX 15 mg/m^2/6hrs PO x 4, wkly x 12 vs MTX 125 mg/m^2/6hrs PO x 4. wkly x 12 + Leucovorin 5 mg/6hrs PO x 6 beginning 36 hours after first dose of MTX	
Chemotherapy	Advanced disease	Neocarzinostatin 3 mg/dx20 IV	Dr. K. Ishii NCI, Tokyo, Japan
Chemotherapy	Advanced disease	5-FU 20 mg/kg IA continuous daily x 10 q 12 wks + CCNU 130 mg/m^2 PO q 6 wks	Dr. R. Elkort Boston University Medical Center
Chemotherapy + Radiation therapy	Unresectable	5-FU 600 mg/m^2/wk IV vs Radiation therapy 4000r + 5-FU 600 mg/m^2 IV d1-3 + 5-FU 600 mg/m^2/wk IV following radiation therapy	Dr. D. J. Klassen University of Ottawa, Canada Eastern Cooperative Oncology Group Protocol
Chemotherapy + Radiation therapy	Curative Resection	No treatment vs 5-FU 500 mg/m^2 IV d1-3 of each radiation course, then wkly for 2 yrs after radiation therapy + Radiation 4000r split course	Dr. Charles Haskel UCLA Medical Center Los Angeles, California GI Tumor Study Group

Chemotherapy + Radiation therapy	Local & unresectable	5-FU 500 mg/m^2 IV d1-3 of each radiation course, then wkly for 2 yrs after radiation therapy + Radiation 6000r split course vs Radiation 6000r split course vs 5-FU 500 mg/m^2 IV d1-3 of each radiation course, then wkly for 2 yrs after radiation therapy + Radiation 4000r split course	Dr. Jacob Lokich Children's Cancer Research Center GI Tumor Study Group
Immunotherapy	Advanced disease	MER-BCG 0.4 mg/wk x 8 ID in each of 5 sites, then q 4 wks (if no progression) vs Q 4 wk schedule x 2, then q 4 wks (if no progression)	Dr. C.G. Moertel Mayo Clinic

Table 3. Colorectal carcinoma.

Modality	Patient Profile	Compared Groups	Investigators
Chemotherapy	Advanced disease of colon or rectum	5-FU 12 mg/kg IV d1-5, followed by 6 mg/kg IV QODx11 vs 5-FU 500 mg/kg d1-4, followed by 500 mg/kg/wk IV vs 5-FU 15 mg/kg/wk IV x 4, followed by 20 mg/kg/wk IV x 4 vs 5-FU 15 mg/kg PO d1-6, followed b6 15 mg/kg/wk PO	Dr. F.J. Ansfield University of Wisconsin Medical Center Central Oncology Group Protocol

Chemotherapy	Curative or palliative resection	No treatment vs 5-FU 12 mg/kg d1-4, followed by 6 mg/kg IV QOD x 5, followed by 12 mg/kg/wk IV	Dr. T. B. Grage University of Minnesota Hospital Central Oncology Group Protocol
Chemotherapy	Curative resection but poor prognosis or palliative (microscopic residual disease)	No treatment vs 5-FU 9 mg/kg IV d1-5 q 7 wks + MeCCNU 120 mg/m^2 PO d1 q 7 wks	Dr. G. A. Higgins, Jr. VA Hospital Washington, D. C. VA Surgical Adjuvant Group Protocol
	Disseminated disease (metastatic)	5-FU 400 mg/m^2/wk IV vs 5-FU 400 mg/m^2/wk IV + MeCCNU 175 mg/m^2 PO q 6 wks	Dr. L. H. Baker Wayne State University School of Medicine Southwest Oncology Group Protocol
	Advanced disease (inoperable or metastatic)	MeCCNU 200mg/m^2 PO q6wks vs MeCCNU 120mg/m^2 PO q 6wks + CTX 600 mg/m^2 IV q 3 wks	Dr. G. Bonnadonna NCI, Milan, Italy
Chemotherapy	Asymptomatic liver metastasis at time of resection	Placebo PO wkly vs 5-FU 750 mg/m^2/wk PO	Dr. F. Muggia Albert Einstein College of Medicine Eastern Cooperative Oncology Group Protocol
Chemotherapy	Inoperable disease refractory to standard therapy	Cycloleucine 300 mg/kg/d x 8 IV infusion followed by 300 mg/kg/wk x 15 IV infusion	Dr. N. Andrews University of California School of Medicine at Davis Central Oncology Group Protocol

Chemotherapy	Inoperable or metastatic disease	5-FU 20 mg/kg/d x 5 PO or 13 mg/kg/d IV q 5 wks vs Adriamycin 60 mg/m^2 IV d1, 22, 43 then q 4 wks (to total of 550 mg/m^2) vs 5-FU 10 mg/kg IV d1-5 & 35-39 q 10 wks + VCR 1 mg/m^2 IV d1&35 q 10 wks + MeCCNU 175 mg/m^2 PO q 10wks	Dr. C. G. Moertel Mayo Clinic
Chemotherapy	Post operative with liver metastasis	5-FU 12 mg/kg IV d1-4, followed by 6mg/kg IV QOD x4, followed by 15 mg/kg/wk IV vs 5-FU Hepatic artery infusion 20 mg/kg/d x 14, reduced to 10 mg/kg/d x 7, followed by 15 mg/kg/wk IV	Dr. W. Shingleton Duke University Medical Center Central Oncology Group Protocol
Chemotherapy	Unresponsive to standard therapy	MTX 60 mg/m^2/wk x 12 IV vs MTX 15 mg/m^2 PO q 6 hrs weekly x 12 vs MTX 125 mg/m^2 PO q 6 hrs x 4, weekly x 12 + Leucovorin 5 mg PO q 6 hrs x6, beginning 36 hrs after first dose of MTX	Dr. C. Vogel Grady Memorial Hospital Atlanta, Georgia Southeastern Cancer Study Group Protocol
Chemotherapy	Curative resection	5-FU IV vs 5-FU intraluminally	Dr. L. M. Rosselot St. Vincent's Hospital New York, New York

Chemotherapy	Advanced disease	5-FU 600mg/m^2/wk IV or PO vs CTX 1000 mg/m^2/d IV q 8 wks + 5-FU 600 mg/m^2/wk PO (wks 2-6) q 8 wks vs MeCCNU 175 mg/m^2 PO q 8 wks vs 5-FU 600 mg/m^2/wk PO + 6-TG 40 mg/m^2/d IV x 14 q 8 wks	Dr. H. Douglass, Jr. Roswell Park Memorial Institute Eastern Cooperative Oncology Group Protocol
Chemotherapy	Curative resection	No treatment vs Mito-C 0.08 mg/kg IV 2x wk x5 vs Mito-C 0.04 mg/kg IV 2x wk (20 mg/50 kg total) + 5-FU 5 mg/kg IV 2x wk (2500 mg/50 kg total) + Ara C 0.4 mg/kg IV 2x wk (200 mg/50 kg total)	Dr. H. Imanaga Aichia Cancer Center Nagoya, Japan Cooperative University Group of Surgical Adjuvant Chemotherapy
Radiation + Surgery	Potentially curable (rectum)	Surgery alone vs Preoperative radiation 4000-4600r + Surgery vs Preoperative radiation 2000-2300R + Surgery	Dr. T. B. Grage University of Minnesota Hospital Central Oncology Group and Radiation Therapy Oncology Group Protocol
Radiotherapy + Chemotherapy	Locally unresectable (rectum)	Radiotherapy 4000-5000r vs Radiotherapy 4000-5000r + 5-FU 600 mg/m^2 IV d1-3 followed by 600 mg/m^2/wk IV after radiation is completed	Dr. D. J. Klaassen University of Ottawa Canada Eastern Cooperative Oncology Group Protocol

Radiation + Surgery	Potentially curable (rectosigmoid)	Surgery alone vs Preoperative radiation 3150r + Surgery	Dr. G. A. Higgins, Jr. VA Hospital, Wash. D. C. VA Surgical Adjuvant Group Protocol
Radiotherapy + Chemotherapy + Surgery	Potentially curable (rectosigmoid)	Preoperative radiation 3450r + Surgery vs Preoperative radiation 3450r + 5-FU 375 mg/m^2 IV d1-4 + Surgery	Dr. A. Gerard St. Pierre University Hospital Brussels, Belgium EORTC Cooperative Group Protocol
Immunotherapy	Advanced disease	MER-BCG 0.4 mg/wk x 8 in each of 5 sites, then q 4 wks (if no progression) vs Q 4 wk schedule x 2, then q 4 wks (if no progression)	Dr. C. G. Moertel Mayo Clinic
Immunotherapy + Radiotherapy	Locally unresectable pelvic disease	Radiation 3000-6000r + BCG by scarification (Tice vs Pasteur) wkly x 4, then q 2 wks x 3 mos vs Radiation 3000-6000r + BCG 25 mg PO q 4 wks x 4, then q 2 wks x 6 vs Radiation 3000-6000r + C-Parvum 4 mg q 2 wks vs 8 mg/d x 7, then 8 mg/wk x 15	Dr. C.G. Moertel Mayo Clinic

APPENDIX B. THE CURRENT STATUS OF TREATMENT STUDIES IN GASTROINTESTINAL CANCER SUPPORTED BY THE NATIONAL CANCER INSTITUTE

I. LARGE BOWEL CANCER

Gastrointestinal adenocarcinomas, in their totality, are the major cancer killers in the United States, accounting for approximately one fourth of all cancer deaths. Large bowel carcinoma (colon and rectum) alone afflicts more patients in the United States than any other malignant disease, excluding skin cancer. In 1971, it was estimated that this tumor would be responsible for 75,000 new cases and 46,000 deaths in the United States.

Carcinoma of the pancreas has become increasingly more prevalent in this country during the past two decades. The rate of increase in reported cases is second only to that of lung cancer and it now ranks fourth as a cause of cancer deaths, exceeded only by cancer of the lung, the large bowel, and breast. An estimated 19,000 Americans developed pancreatic carcinoma in 1973 and the same number died of this neoplasm.

Until recently, carcinoma of the stomach was the most common cause of cancer deaths in the United States. The steady decline in its incidence is reflected in a continuing downward trend in the age-adjusted mortality rate per 100,000 population from 28.9 deaths in 1930 to 9.7 deaths in 1967. Nevertheless, it still ranks seventh in the cancer mortality rate for both sexes in this country.

Treatment programs in gastrointestinal cancer are funded by various areas of the National Cancer Institute and it is the purpose of this paper to review these programs and the protocol studies currently sponsored.

The Division of Cancer Treatment has developed a strategy for integrating chemotherapy into a combined modality approach for all major solid tumors. This involves testing of new drugs and regimens in the advanced disease population in order to elucidate the optimal cell kill potential of chemotherapy. Then, chemotherapy will be combined with surgery and/or radiotherapy for the treatment of local and regional disease in the hope of increasing the cure rate. In line with this strategy, the studies for each of the major gastrointestinal cancers will be reviewed, beginning with investigation of chemotherapy in advanced disease and moving to the protocols for local disease that employ surgery and radiotherapy either alone or in combination.

LARGE BOWEL CANCER

Large bowel cancer studies are funded in both the Division of Cancer Trea ment and the Division of Cancer Research Resources and Centers and cover th range from advanced disease through regional disease to local disease (Table

Advanced Disease Protocols

The Mayo Clinic is currently performing a comparative Phase II study of combined 5-FU + methyl CCNU + vincristine *vs* 5-FU + CAC platinum *vs* ICRF-159 (Figure 1). In a previous study, the combination of methyl CCNU, 5-FU, and vincristine showed superiority in objective response rate over that of a control arm of 5-FU alone. Only a small number of patients were treated with t combination, so an extended Phase II trial seemed indicated. Eventually it is planned to compare the combination of 5-FU and methyl CCNU with or without vincristine.

In several tumor systems, CAC platinum has shown a potentiating effect when combined with 5-FU. A pilot study at Mayo Clinic revealed that low dose of the platinum drug increase the frequency, severity, and duration of 5-FU induced luekopenia. Now that an appropriate dose level for the combination has been determined, it will be evaluated for therapeutic efficacy. ICRF-159 is a new drug not previously evaluated against large bowel cancer.

The Mayo Clinic is also performing a clinical evaluation of MER (methanol extraction residue) therapy in patients with advanced gastrointestinal cancer, including large bowel. MER, a methanol extraction residue of BCG, is a non-viable material that has been extensively studied in Israel by Weiss and his associates. This study will investigate the local reactions and systemic toxicity of MER, its effect on immunologic determinants, and the therapeutic effect in patients with advanced metastatic gastrointestinal carcinoma.

The NCI in Milan is currently emgaged in a controlled study of methyl CCNU + cyclophosphamide compared to methyl CCNU alone in advanced carcinoma of the large bowel and stomach (Figure 2). The University of Leeds has just completed an evaluation of methyl CCNU and is currently planning their next regimen for study.

The Eastern Cooperative Oncology Group (ECOG) has completed a study (#0870) screening drugs which had received little prior testing in colon cancer. Five drugs were tested according to the following schema:

Streptozotocin	0.5 gm/m^2/wk IV
CCNU	130 mg/m^2 single dose PO
Bleomycin	20 mg/m^2 2 x wk IV
6-Thioguanine	1.0 mg/kg/d PO
Procarbazine	100 mg/d x 7 PO

The results revealed a low, but definite, incidence of remission with 6-thioguanine and CCNU but the other drugs lacked activity. The current ECOG study (Figure 3) is evaluating 5 new regimens in advanced disease; at the time of disease progression, patients are crossed over to 3 of these regimens and to a new drug (β-2'-deoxythioguanosine).

The Southwest Oncology Group has a current study (SOG 7302) intended to compare the effectiveness of 5-FU + methyl CCNU vs 5-FU alone in the treatment of disseminated large bowel cancer. The 5-FU will be given weekly without loading course (Figure 4).

A Central Oncology Group protocol (#7030) is comparing the incidence, quality, and duration of regression, and survival produced in large bowel cancer by 5-FU given on 4 different schedules (Figure 5). This study will compare two "toxic" schedules (loading course IV and weekly IV) with a "non-toxic" schedule and an oral schedule.

The Department of Developmental Therapeutics of M. D. Anderson Hospital has recently evaluated a regimen termed FCC (5-FU, Cytoxan, and CCNU) that has significant activity in colon carcinoma. Currently, the group is investigating Ftorafur at a dosage of 1 gm/m^2/d x 5 IV q 2-3 wks for all patients who have not received 5-FU.

The ECOG is about to begin a protocol (#3273) involving a double-blind comparison of oral 5-FU vs placebo following resection of large bowel cancer having asymptomatic liver metastases (Duke's D) (Figure 6). The Duke's D (distant metastases, peritoneal seeding, parietal or adjacent organ invasion) stage of large bowel cancer is found in 30% of all patients presenting with this tumor. Liver metastases, in particular, are found in about 10% of patients and, when present at the time of initial resection, the prognosis is uniformly dismal. Two year survival rates range from 4-18% and the median survival varies from 4-10 months. This study will attempt to determine whether oral 5-FU given immediately after the discovery of asymptomatic liver metastases will increase the symptom free interval, the time to overt disease progression, and, ultimately, the survival in this group when compared to placebo.

The Central Oncology Group is also evaluating 5-FU in a similar therapeutic situation, but is comparing intraarterial infusion of the drug versus the standard intravenous usage (Figure 7). Intraarterial infusion chemotherapy has been of interest since the appearance of the reports by Sullivan and his coworkers. In 1965, Sullivan and Zurek reported an objective response rate of approximately 60% in patients with advanced liver metastases treated by a protracted ambulatory infusion of 5-FUDR. In a more recent report, Watkins et al. summarized data on 108 patients treated in this manner with 60 showing evidence of a favorable response to therapy. The median survival time in this group was 15 months compared to 4.2 months for 80 patients of a matched control group. The objectives of the COG study are to compare the effect on response rate and survival produced by sustained systemic chemotherapy with 5-FU vs hepatic artery infusion of 5-FU, followed by sustained systemic 5-FU in selected patients with large bowel adenocarcinoma.

The Southeastern Cooperative Study Group is beginning a protocol that will study methotrexate on 3 schedules in large bowel cancer. The drug will be administered on the following regimens:

1. 60 mg/m^2 IV weekly x 12
2. 15 mg/m^2 PO q 6 hrs x 4, weekly x 12
3. 125 mg/m^2 PO q 6 hrs x 4, weekly x 12

   +

   Leucovorin 5 mg PO q 6 hrs x 6 beginning 36 hrs after
   the first dose of methotrexate

At the Memorial Sloan-Kettering Cancer Center, Krakoff and Golbey are studying at a 3-drug regimen called "MFC" consisting of:

Mitomycin C	0.06-0.08 mg/kg	IV 2 x wkly till toxicity, then weekly
5-FU	7.5-10 mg/kg	
Ara-C	0.8-2 mg/kg	

Ota et al. from Japan had described a high response rate in colon cancer (60%) using this regimen, but useful response at Memorial Hospital has occurred in only 3 of 31 colon cancer patients.

Other groups, including Acute Leukemia Group B and the Western Grou have drug-oriented studies that include large bowel cancer patients among groups of either adenocarcinomas or mixed solid tumors. These studies will not be described since they have not been designed from a disease-oriented standpoint, although this does not imply that valuable data might not result from such investigations.

To summarize the status of studies in advanced large bowel cancer, 2 DCT contractors, 4 cooperative groups, and 2 grant supported comprehensiv cancer centers are currently evaluating 14 single drug regimens, five 2-drug regimens, and three 3-drug regimens. Six of the single agent studies are 5-FU on six different regimens differing either by schedule, route of administration, or dosage level (Table 2). Four of the 2-drug regimens (Table 3) and all of of the 3-drug regimens (Table 4) employ 5-FU, highlighting the ubiquitousness of this drug in trials against large bowel cancer.

Regional Disease

Radiation therapy has been the treatment of choice in regional or locally unresectable disease while the addition of drugs, such as 5-FU, has been attempted.

In an early controlled study, the Mayo Clinic compared radiotherapy plu 5-FU against radiotherapy + placebo in locally unresectable patients whose known malignant disease could be encompassed by a radiation field no larger than 20 x 20 cm. Each patient received 900-1200 rads per 6 day week to a total dose of 3500-4000 rads, with supervoltage equipment. 5-FU was given in a total dose of 40-50 mg/kg at the onset of radiation. The mean survival was 16.8 months in 33 patients receiving the x-ray alone compared to 22.8 months in 32 patients who also were given the 5-FU ($P < 0.05$). In addition, the mean duration of symptomatic control was longer in the 5-FU treated group althoug both groups had an equal number of patients achieving symptomatic control. A follow-up study supported by the grant mechanism (PO2 CA11911) is comparing 5-FU alone, 100% oxygen inhalation alone, and 5-FU plus 100% oxygen inhalation as adjuvants to radiation therapy of locally unresectable carcinoma.

The ECOG recently began a study of locally unresectable stomach, pancreatic, rectal, and biliary tract cancer (Figure 8). This study will evaluate the effectiveness of 5-FU, as both an adjuvant to radiation therapy and as weekly maintenance versus radiation therapy alone for the locally unresectabl rectal lesions. Radiotherapy for the rectal lesions will consist of 4000-5000 rads in fractions of 200 rads given 5 days a week using either 2 parallel opposed fields, 3 fields, or rotation techniques. The field size is not to exceed 20 x 20 cm and the source will be cobalt 60 or a similar high energy form.

Local Disease

Local disease studies currently in progress involve two major approaches: (1) systemic chemotherapy administered after surgical resection, and

(2) preoperative radiotherapy in patients with rectal carcinoma.

Chemotherapy and Surgery. The rationale of employing chemotherapy as a surgical adjuvant has evolved significantly over the past 15 years. Initially, the use of chemotherapeutic agents was limited to an interoperative or immediate postoperative course in an attempt to sterilize the clonigenic cells liberated by surgical trauma. This empirical approach was based on early observations of circulating tumor cells and on experimental work that noted the beneficial antitumor activity against these cells resulting from chemo therapy developed in animal model systems. However, little consideration was given to the growth kinetics of bowel tumor primaries or metastases, and the available drugs used had only a relatively low order of activity in advanced bowel cancer.

Current studies, which largely involve 5-FU, employ chemotherapy administered for a longer period of time in the hope of eradicating the microscopic foci of metastatic disease that remain after surgery. The Veterans Adinistration Surgical Adjuvant Cancer Chemotherapy Study Group has recently ceased patient entry on a study (Protocol 23) of the effect of prolonged intermittent chemotherapy on the survival of male patients following resection for large bowel cancer (Figure 9). This study employed 5-FU in 5-day courses starting 14 days after surgery and repeated at 6 week intervals for 18 months in patients having curative resections with a poor prognosis for cure. The criteria for the poor prognosis group included:

1. Positive lymph nodes
2. Serosal penetration or invasion of peri-rectal fat
3. Blood vessel invasion
4. Lymphatic invasion
5. Cancer in other organs removed as part of the resection.

Additionally, drug was administered to patients with a proven palliative resection (defined as removal of the primary lesion, but with microscopic evidence of residual disease, i. e., cancer at a resection margin or biopsy of tissues not removed in the resection). Although analysis of this study is not complete at this time, it does not appear that 5-FU significantly increased the survival of patients in comparison to the untreated control group.

In their current study, which began in July, 1973 (Protocol 27), the VA group will follow the same experimental design but the chemotherapy will be a combination of methyl CCNU + 5-FU. The drugs will be administered in 5 day courses, beginning as soon after surgery as the patient's condition permits (about 10-14 days after operation), and repeated at 7 week intervals for one year. On the first day, oral methyl CCNU will be given in a dose based on 120 mg/m^2 and, on the same day and each of the next four days, 5-FU will be given at 9 mg/kg IV.

The Central Oncology Group has initiated a study (#7041) that also attempts to determine the effect of long-term adjuvant chemotherapy with 5-FU on the rate of recurrence, time of recurrence, and survival in selected patients treated surgically for carcinoma of the colon and rectum (Figure 10). As in the VA study, two groups will be treated (i. e., a curative resection group with poor prognosis for cure and a palliative resection group). The COG defines these groups similarly to the VA group. The chemotherapy differs in that 5-FU is given in a single intensive course within one month of surgical resection and is followed by weekly maintenance therapy for one yea

In the area of traditional project grants, a cooperative study headed by Dr. L. M. Rousselot of St. Vincents Hospital in New York is being funded. The group includes several hospitals in the New York city area. Their study will compare 5-year survival obtained by the intraluminal use of 5-FU versu 5-FU given IV at the time of surgery and thereafter. This study is based on Rousselot's published data showing that 5-FU (30 mg/kg in 50 cc intraluminal at surgery plus 10 mg/ kg IV for 2 days thereafter gave a 74% 5 year survival in 60 patients with stages I and II disease and a 65% 5-year survival for 21 patients with stage III disease.

Preoperative Radiotherapy. Rectal cancer has a poorer prognosis than l sions located higher in the colon. The overall survival for 798 cases of rectal carcinoma treated at the University of Minnesota hospitals was 27% for the period 1940-1950 and 30% for the period 1951-1959. The resectability rate rose from 76% for the first period to 85% in the second period.

Numerous investigators have explored the use of radiation therapy in the treatment of carcinoma of the rectum, either as an adjuvant to surgical resection or for palliation. With the advent of modern megavoltage therapy and increased knowledge of radiobiology, substantial progress has been made in the radiotherapy of rectal carcinomas.

The largest experience with preoperative radiation therapy as a surgical adjuvant in rectal cancer has been reported by the Memorial Hospital for Cancer and Allied Diseases. Between 1939 and 1951, 1700 cases of rectal cancer were treated preoperatively with radiation therapy at dose levels between 150 and 2000 rads, via two anterior and two posterior pelvic ports using the 250 KV machine. An additional perineal port was included for tumors within 10 cm of the anal verge. The 5-year survival and 10-year survival figures were comparable in patients with Duke's A and B lesions, who had received radiation therapy, and in patients who had not received radiation therapy. However, among patients having spread of tumor to the regional nodes, 37% of those who received radiation therapy were alive at 5-years and 27% were alive at 10 years; by comparison, in the non-irradiated group only 23% and 10% survived 5 and 10 years, respectively. It should be emphasized that the decision on which patients received radiation and which ones did not was not made by random allocation.

A subsequent study with carcinoma of the rectum, which randomized 700 patients from the same institution into preoperative radiation therapy plus surgery or surgical resection alone, revealed a reversal of the figures. In the radiotherapy group, 40% survived 5 years vs 47% in the non-irradiated group with nodal spread of cancer.

A prospective randomized and controlled study to determine the clinical effect of low dose preoperative irradiation on clinically operable patients with adenocarcinoma of the rectum and rectosigmoid was initiated by the VA Surgical Adjuvant Group in 1964. A preliminary analysis using life table procedures to produce survival curves, because only a portion of the patient population has been followed for 5 years, reveals the following results. Of 199 patients who had a resection other than combined abdomino-perineal resection, the 5-year survival for the treated group was 40% compared to 45% in the non-irradiated group. However, in 414 patients undergoing combined abdomino-perineal resection the 18 month survival was 74% in the irradiated group and 64% in the control group; the 5-year survival figure is 46% for the treated group and 30% for the controls. Further analysis of the data reveals no particular benefit in those patients who have positive regional nodes. However, positive lymph nodes were found in only 27% of the specimens resected in patients who received preoperative radiation therapy as compared with 40% in the control group.

Radiation therapy consisted of a tissue dose of 2000 rads given in 10 increments of 200 rads over a 2-week interval delivered to the mid-plane of the pelvis through opposing anterior-posterior portals. Either conventional beam therapy or high energy therapy was used depending on which was available in the individual hospital. When the tumor was within 8 cm of the anal verge, an additional 500 rads was administered in 50 rad increments through a perineal portal during the same period. Operation was carried out as soon as feasible following treatment.

At the present time five major studies are in progress using high dose radiation therapy in carcinoma of the rectum and rectosigmoid. At the University of Oregon Medical School, 5000 rads by megavoltage irradiation have been given to 52 patients with rectosigmoid carcinoma. There was no control group. Patient tolerance was good although there was some diarrhea in a small percentage of patients. A preliminary analysis indicates that no residual gross tumor was found in 10 of 51 patients who completed irradiation and resection. Positive lymph nodes were found in 10 of the 51 cases. There has been no clinical evidence of local recurrence at the primary site in 51 patients. At the time of publication of their report, Allen and Fletcher found 28 of these 51 patients alive without evidence of disease. The study was not randomized and it is impossible to assess the long-term survival benefits with the addition of preoperative irradiation.

Another study is being conducted at the Yale-New Haven Hospital, by Kligerman, Hayes, Vidone and their associates. Patients with potentially curable adenocarcinoma of the rectum and rectosigmoid were randomized by a "flip of the coin" method. Patients in the treatment group received 4400-4600 rads from a 6 MEV linear accelerator over a period of 4-1/2 weeks. The irradiation field employed anterior and posterior portals and included the primary tumor, adjacent tissue, and the lymph node drainage area in the true pelvis as well as that extending along the superior hemorrhoidal vessels to the level of the second lumbar vertebra. The radiotherapy was well tolerated, although a high incidence of diarrhea was reported during treatment. Surgical resection was carried out 4 weeks after completion of the radiation therapy. No significant increase in the difficulty of performing the surgical resection or in postoperative morbidity and mortality is reported. Of the 13 patients randomized to receive preoperative radiation, one died in the postoperative period; none of the 16 patients treated by resection alone died in the postoperative period.

The number of patients is too small, and the follow-up period too short, to permit meaningful information regarding long-term survival. However, examination of the resected specimens revealed that 11 of the 16 patients randomized to surgical resection alone had tumor involvement of the regional lymph nodes; only 3 of 13 patients treated by preoperative irradiation had positive nodes.

The cooperative clinical groups are currently performing 3 major studies in this area (Table 5). The Central Oncology Group is evaluating two dose levels of preoperative radiotherapy (4000-4600 rads and 2000-2300 rads) vs an untreated control. The VA group is now studying an intermittent dose of 3150 rads, while the ECOG is evaluating 4000-5000 rads with and without 5-FU, without an untreated control group.

The EORTC Gastrointestinal Tumor Group is employing 3450 rads with and without 5-FU, again without an untreated control.

Conclusion

This review has described or briefly mentioned 21 current investigations of treatment approaches in large bowel cancer which are supported by the NCI (Table 6). The list may not be encyclopedic for all efforts that are supported by the NCI, especially in the traditional project grants and comprehensive centers area. It is clear, however, that treatment research is spread diffusely through two of the four NCI divisions by at least 4 different funding and administrative approaches. In addition, there is the Large Bowel Cadre that is yet to develop any funded thrust in the treatment area.

The DCT plans a program expansion through combined modality studies for regional and local disease to be developed by the present Gastrointestinal Tumor Study Group and in specific new contracts in the advanced disease area. The major problem faced by the NCI is the coordination of all of this research to insure maximum efficiency in the studies without unintended overlap. What is both lacking and needed is an overall strategy guiding all studies so that priorities of funding, regardless of the mechanism, can be established to reac the goal of increased cure rates as rapidly as possible.

Table 1. NCI supported resources in large bowel cancer.

Group	Type of Support	NCI Division*	Disease Stage Under Treatment† Advanced Disease	Regional Disease	Local Disease
I. Phase II Chemotherapy Studies	Contract	DCT			
1. Mayo Clinic			X	X	
2. NCI, Milan			X		
3. University of Leeds			X		
II. Gastrointestinal Tumor Study Group	Contract	DCT	ⓧ	ⓧ	ⓧ
1. Mayo Clinic					
2. Roswell Park					
3. Harvard					
4. Yale					
5. U.C.L.A.					
6. U.S.C.					
7. University of Miami					
III. VA Surgical Adjuvant Study Group	Transfer of funds	DCT			X
IV. Cooperative Groups	Contract	DCRRC			
1. Eastern Cooperative Oncology Group			X	X	X
2. Central Oncology Group			X		X
3. Southwest Oncology Group			X		
4. Acute Leukemia Group B			X		
5. Western Cancer Study Group			X		
6. Radiation Therapy Oncology Group					X
7. Southeastern Cancer Study Group			X		
V. National Large Bowel Cancer Project M. D. Anderson Hospital	Grants	DCRRC			X
VI. Program Projects & Cancer Centers	Grants	DCRRC	X	X	X
VII. Traditional Project Grants	Grants	DCRRC			X

† X = Studies in progress
ⓧ = Planned studies

*DCT = Division of Cancer Treatment
DCRRC = Division of Cancer Research Resources & Centers

Table 2. Single agent regimens under evaluation in large bowel cancer.

Drug	Dose Schedule	Group
ICRF-159	100 $mg/m^2/d$ x 3	Mayo Clinic (DCT contract)
Methyl CCNU	75 mg/m^2 PO q 8 wks	ECOG 4273
Methyl CCNU	200 mg/m^2 PO q 6 wks	NCI, Milan (DCT contract)
5-FU	600 $mg/m^2/wk$ IV or PO	ECOG 4273
5-FU	400 $mg/m^2/wk$	SWG 7302
5-FU	12 mg/kg/d x 4-5; then, 6 mg/kg QOD until toxicity	COG 7030
5-FU	15 mg/kg/wk IV	COG 7030
5-FU	500 mg/d x 4 IV; then, 500 mg/wk	COG 7030
5-FU	15 mg/kg/d x 6 PO; then, 15 mg/kg/wk PO	COG 7030
Ftorafur	1 $gm/m^2/d$ x 5 IV q 2-3 wks	M. D. Anderson
β-2'-deoxythioguanosine	100 $mg/m^2/d$ x 5 IV q 3 wks	ECOG 4273
Methotrexate	60 $mg/m^2/wk$ x 12 IV	SEG
Methotrexate	15 mg/m^2 PO q6h x 4, wkly x 12	SEG
Methotrexate	125 mg/m^2 PO q6h x 4, wkly x 12 + Leucovorin 5 mg PO q6h beginning 36 hrs after first dose of MTX	SEG

Table 3. 2-Drug combination regimens being evaluated in large bowel cancer.

Drug Regimens	Group
5-FU (12 mg/kg/d x 5 IV) + Cis-platinum (20 mg/m^2/d x 5 IV)	Mayo Clinic (contract)
Methyl CCNU + Cytoxan (600 mg/m^2 IV q 3 wks)	NCI, Milan (contract)
Cytoxan (1 gm/m^2 d1) + 5-FU (600 mg/m^2/wk, d 8, 15, 22, 29 PO) Repeat courses q 8 wks	ECOG 4273
5-FU (600 mg/m^2/wk PO) + 6-Thioguanine (40 mg/m^2/d x 14) Repeat courses q 8 wks	ECOG 4273
Methyl CCNU (175 mg/m^2 PO q 6 wks) + 5-FU (400 mg/m^2/wk IV)	SOG 7302

Table 4. 3-Drug combination regimens being evaluated in large bowel cancer

Drug Regimens		Group
5-FU (10 mg/kg/d x 5) + Methyl CCNU (175 mg/m^2 PO, d 1) + Vincristine (1 mg/m^2)		Mayo Clinic (contract)
5-FU + Cytoxan + CCNU		M. D. Anderson
Mitomycin C (0.06-0.08 mg/kg) 5-FU (7.5-10 mg/kg) Ara-C (0.8-2 mg/kg)	IV 2x weekly until toxicity. Then weekly	Memorial Hospital-Sloan Kettering Cancer Center

Table 5. Preoperative radiotherapy studies in rectosigmoid cancer.

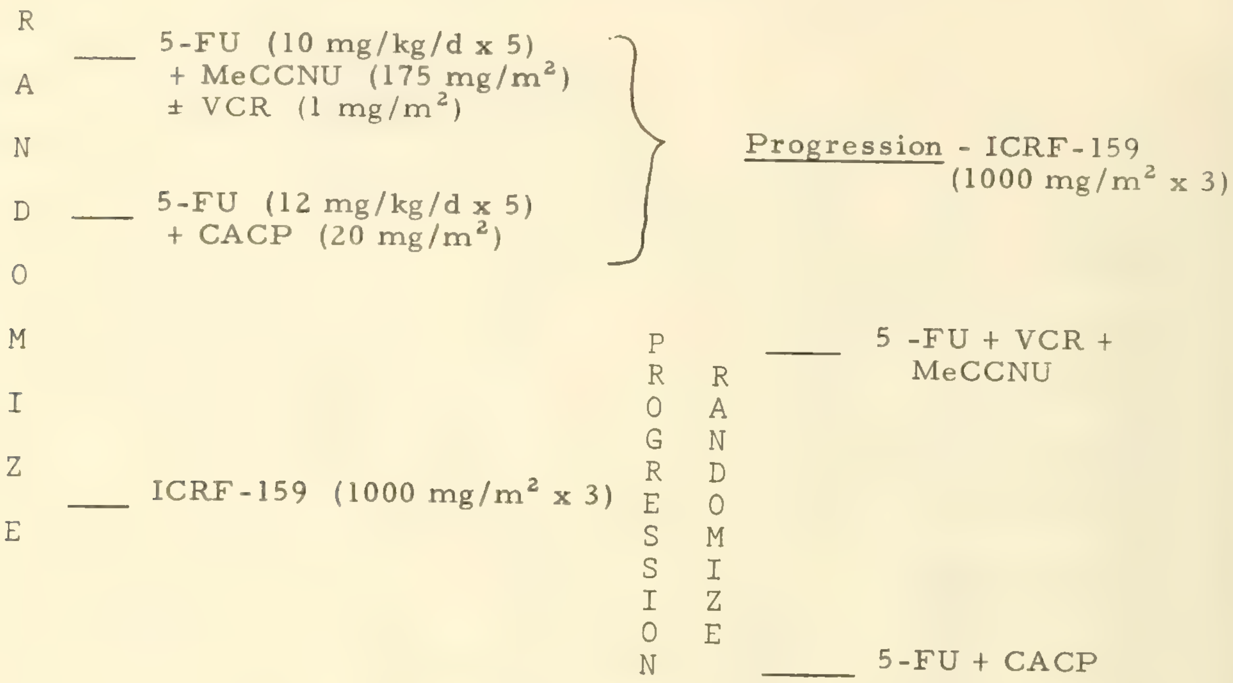
Group	Patient Profile	Compared Groups				
Dr. T. B. Grage University of Minnesota Hospital Central Oncology Group and Radiation Therapy Oncology Group Protocol	Potentially curable (rectum)	Surgery alone	vs	Preoperative radiation (4000-4600R) + Surgery	vs	Preoperative radiation (2000-2300R) + Surgery
Dr. G. A. Higgins, Jr. V. A. Hospital Washington, D. C. V. A. Surgical Adjuvant Group Protocol	Potentially curable (rectosigmoid)	Surgery alone	vs	Preoperative radiation (3150R) + Surgery		
Dr. D. J. Klaassen University of Ottawa Canada Eastern Cooperative Oncology Group Protocol	Locally unresectable (rectum)	Radiotherapy (4000-5000R)	vs	Radiotherapy (4000-5000R) + 5-FU 600 mg/m^2/d x 3 IV followed by 600 mg/m^2/wk IV after radiotherapy is completed		
Dr. A. Gerard St. Pierre University Hospital Brussels, Belgium EORTC Cooperative Group Protocol	Potentially curable (rectosigmoid)	Preoperative radiotherapy (3450R) + Surgery	vs	Preoperative radiation (3450R) + 5-FU 375 mg/m^2/d x 4 IV + Surgery		

Table 6. Breakdown by disease stage of 21 studies in large bowel cancer supported by the NCI.

Supporting NCI Area	Disease Stage Advanced Disease	 Regional Disease	 Local Disease	Total
Division of Cancer Treatment (contracts or transfer of funds)	2	1	2	5
Division of Cancer Research Resources & Centers (cooperative groups)	4	1	5	10
Division of Cancer Research Resources & Centers (comprehensive centers)	2	1	2	5
Project Grants	-	-	1	1
Totals	8	3	10	21

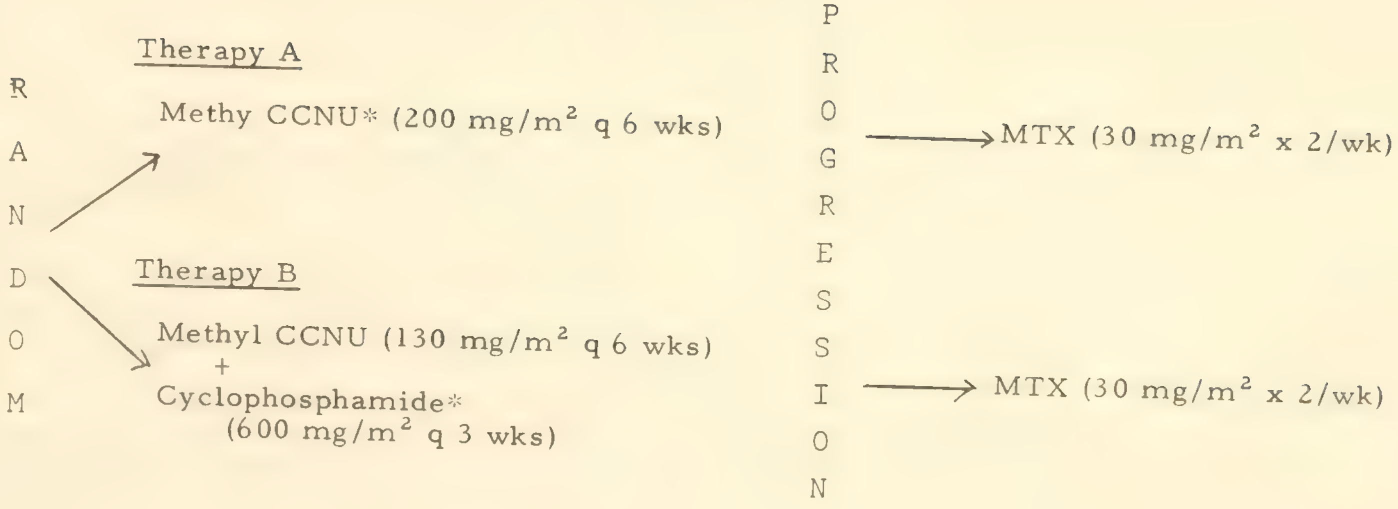
Including EORTC

I. Untreated patients - or prior Rx with agents other than 5-FU, VCR, MeCCNU or CACP



II. Patients previously treated with 5-FU, VCR, MeCCNU or CACP Rx.: ICRF-159

Figure 1. Mayo Clinic comparative Phase II study in large bowel cancer.



Age	CCNU*	Cyclophosphamide*
>65	110 mg/m²	400 mg/m²
<65	130 mg/m²	600 mg/m²

Figure 2. NCI, Milan, study of advanced gastrointestinal cancer.

SCHEMA

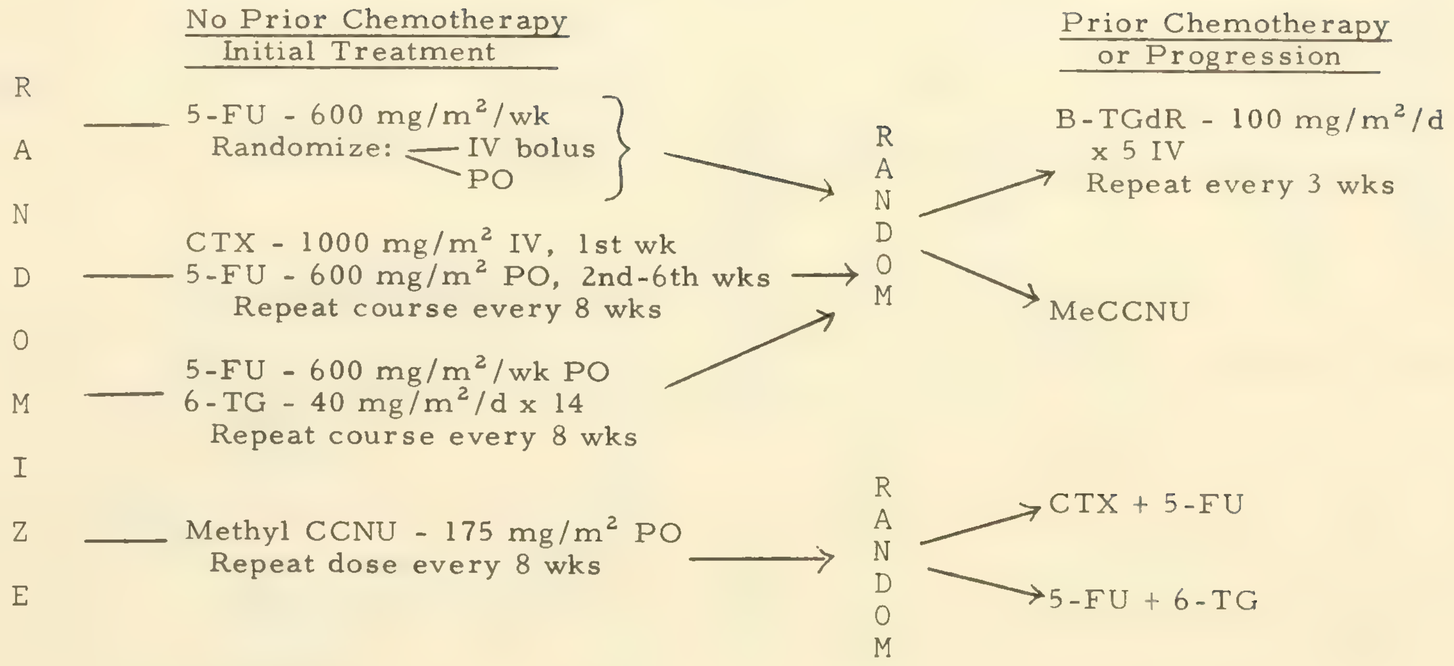


Figure 3. ECOG 4273 - Chemotherapy of colon and rectal carcinoma (Phase II and III).

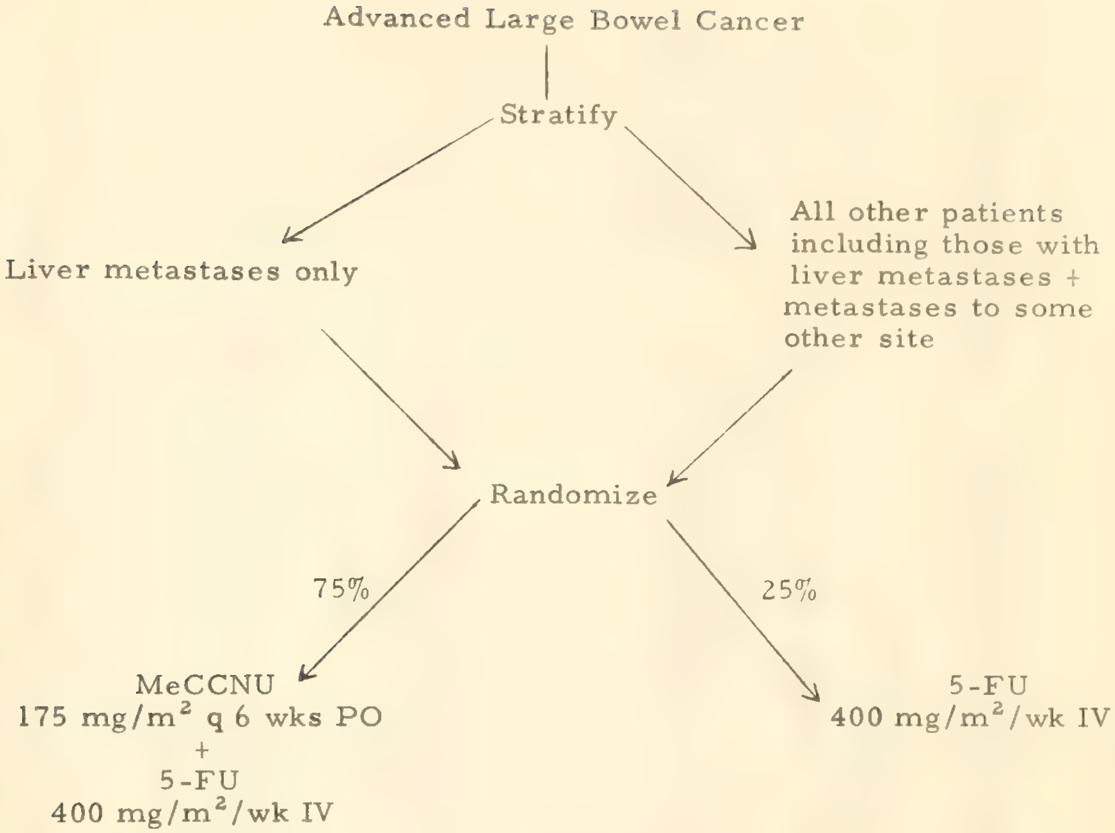

Advanced Large Bowel Cancer
Stratify
Liver metastases only
All other patients including those with liver metastases + metastases to some other site
Randomize
75%
25%
MeCCNU
175 mg/m² q 6 wks PO
+
5-FU
400 mg/m²/wk IV
5-FU
400 mg/m²/wk IV

Figure 4. Southwest Oncology Group Protocol 7302.

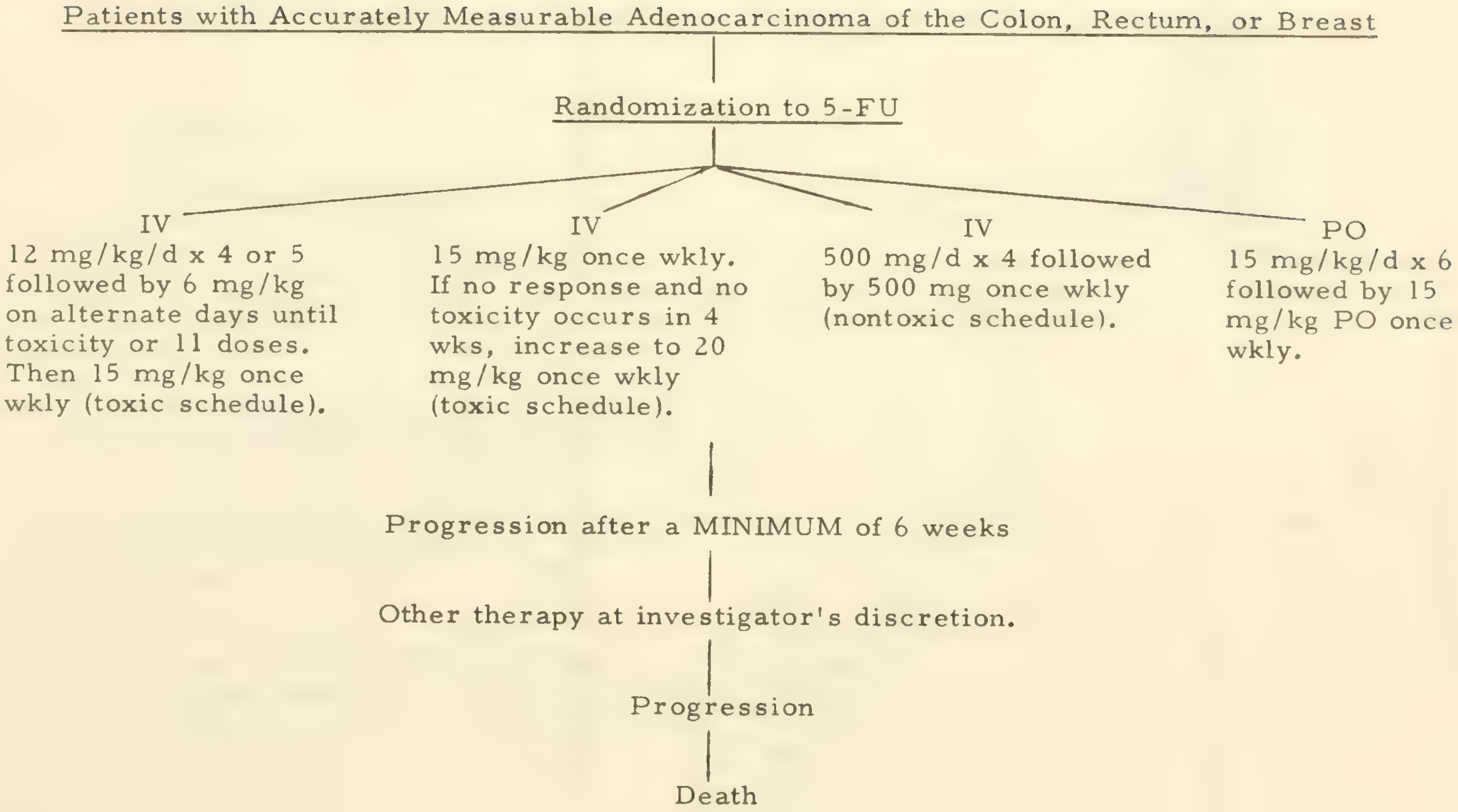

Patients with Accurately Measurable Adenocarcinoma of the Colon, Rectum, or Breast
Randomization to 5-FU
IV
12 mg/kg/d x 4 or 5 followed by 6 mg/kg on alternate days until toxicity or 11 doses. Then 15 mg/kg once wkly (toxic schedule).
IV
15 mg/kg once wkly. If no response and no toxicity occurs in 4 wks, increase to 20 mg/kg once wkly (toxic schedule).
IV
500 mg/d x 4 followed by 500 mg once wkly (nontoxic schedule).
PO
15 mg/kg/d x 6 followed by 15 mg/kg PO once wkly.
Progression after a MINIMUM of 6 weeks
Other therapy at investigator's discretion.
Progression
Death

Figure 5. Central Oncology Group Protocol 7030: Phase III study of 4 doses of 5-FU.

SCHEMA

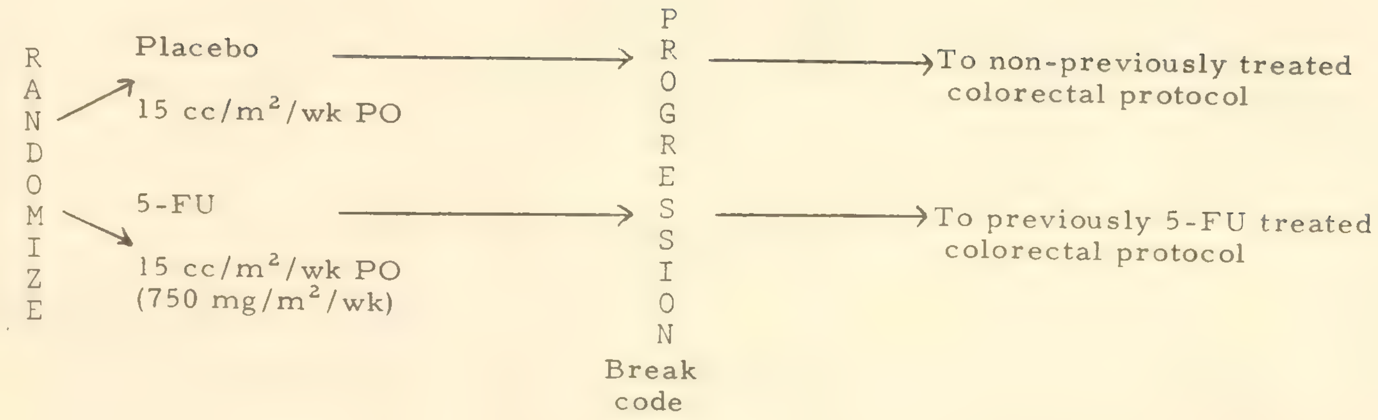
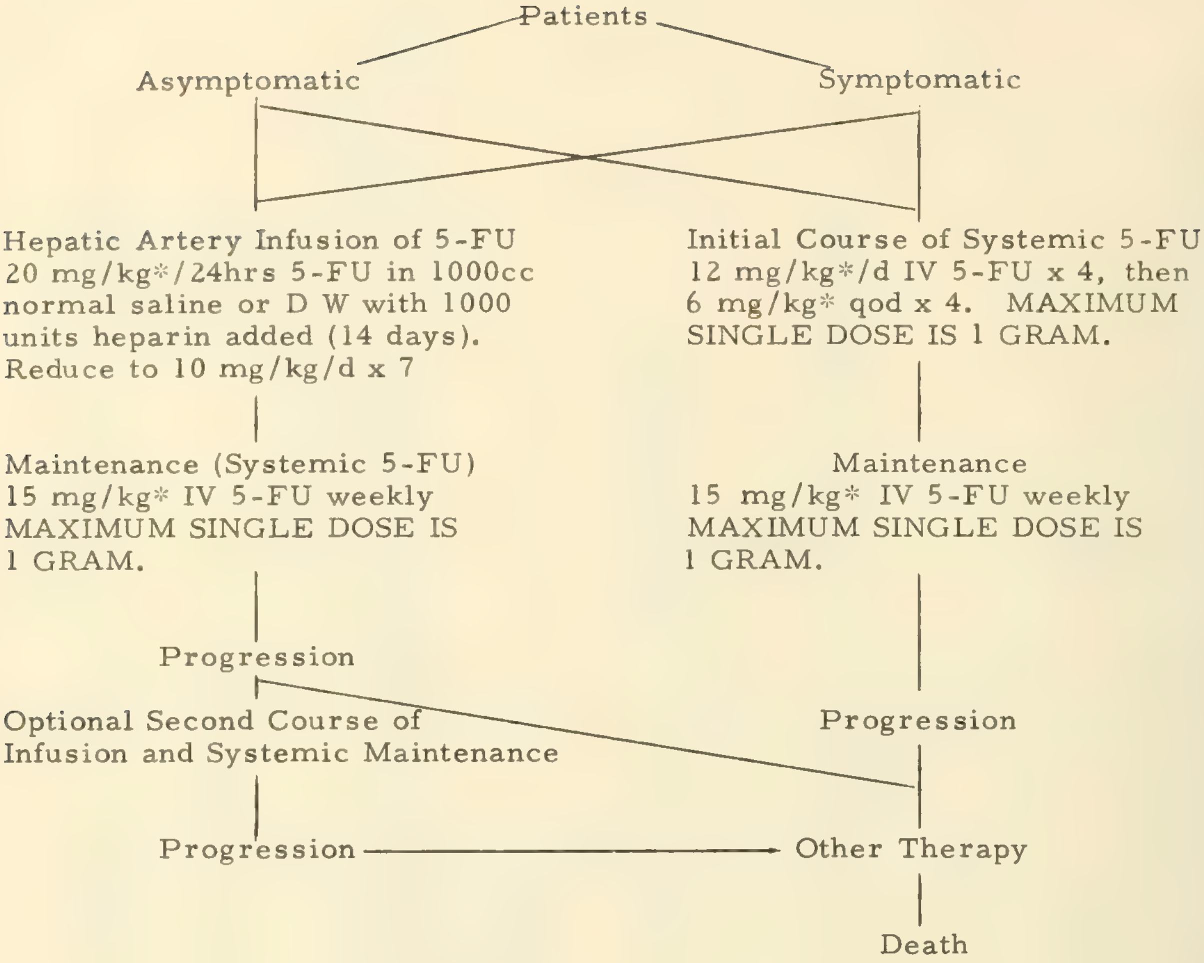

RANDOMIZE
Placebo
15 cc/m²/wk PO
5-FU
15 cc/m²/wk PO
(750 mg/m²/wk)
PROGRESSION
Break code
To non-previously treated colorectal protocol
To previously 5-FU treated colorectal protocol

Figure 6. ECOG protocol 3273.



Hepatic Artery Infusion of 5-FU
20 mg/kg*/24hrs 5-FU in 1000cc normal saline or D W with 1000 units heparin added (14 days). Reduce to 10 mg/kg/d x 7

Initial Course of Systemic 5-FU
12 mg/kg*/d IV 5-FU x 4, then 6 mg/kg* qod x 4. MAXIMUM SINGLE DOSE IS 1 GRAM.

Maintenance (Systemic 5-FU)
15 mg/kg* IV 5-FU weekly
MAXIMUM SINGLE DOSE IS 1 GRAM.

Maintenance
15 mg/kg* IV 5-FU weekly
MAXIMUM SINGLE DOSE IS 1 GRAM.

Optional Second Course of Infusion and Systemic Maintenance

*Based on the lesser of actual and ideal weights.

Figure 7. COG study in patients with hepatic metastases from adenocarcinoma of colon or rectum.

SCHEMA

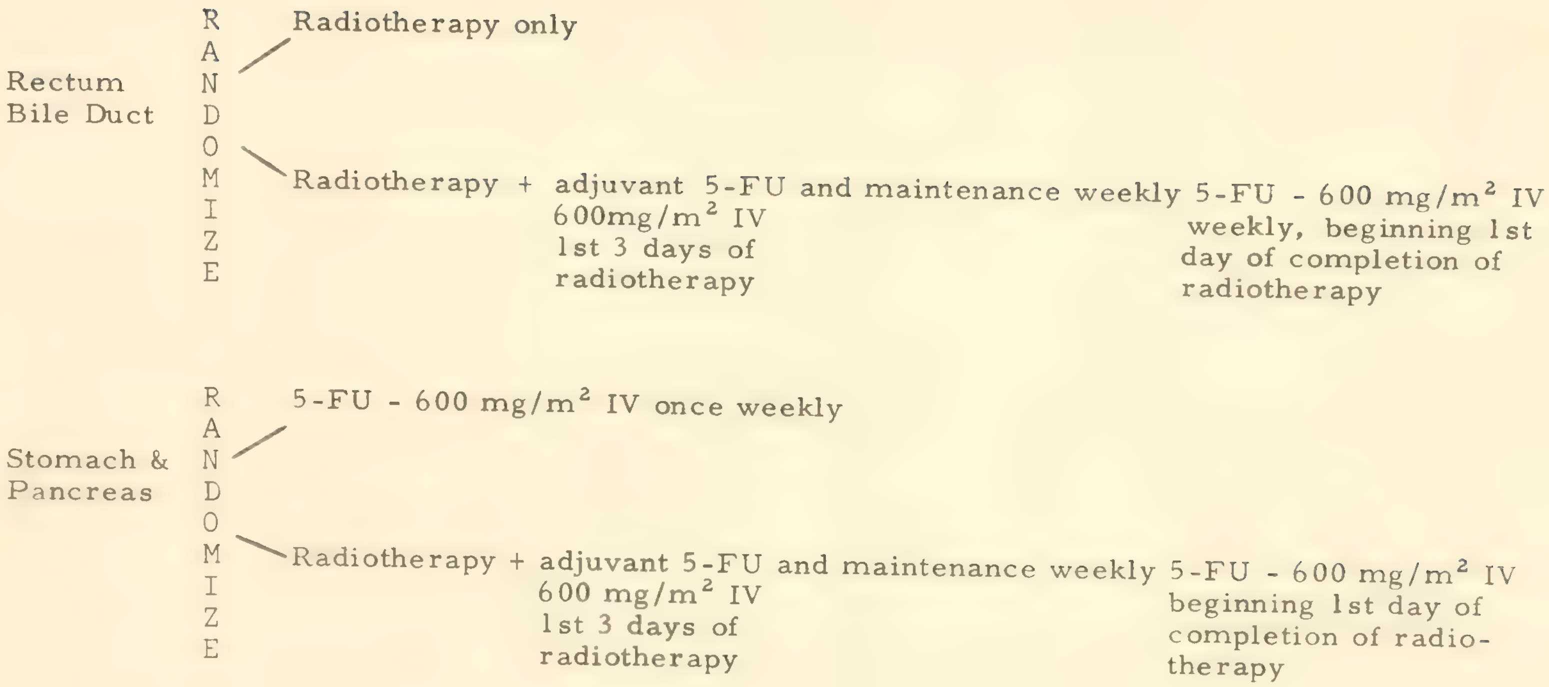

R A N D O M I Z E
Radiotherapy only
Rectum
Bile Duct
Radiotherapy + adjuvant 5-FU 600mg/m² IV 1st 3 days of radiotherapy and maintenance weekly 5-FU - 600 mg/m² IV weekly, beginning 1st day of completion of radiotherapy
R A N D O M I Z E
5-FU - 600 mg/m² IV once weekly
Stomach &
Pancreas
Radiotherapy + adjuvant 5-FU 600 mg/m² IV 1st 3 days of radiotherapy and maintenance weekly 5-FU - 600 mg/m² IV beginning 1st day of completion of radiotherapy

Figure 8. ECOG study (1274) of treatment in locally unresectable stomach, pancreatic, rectal, and biliary tract cancer.

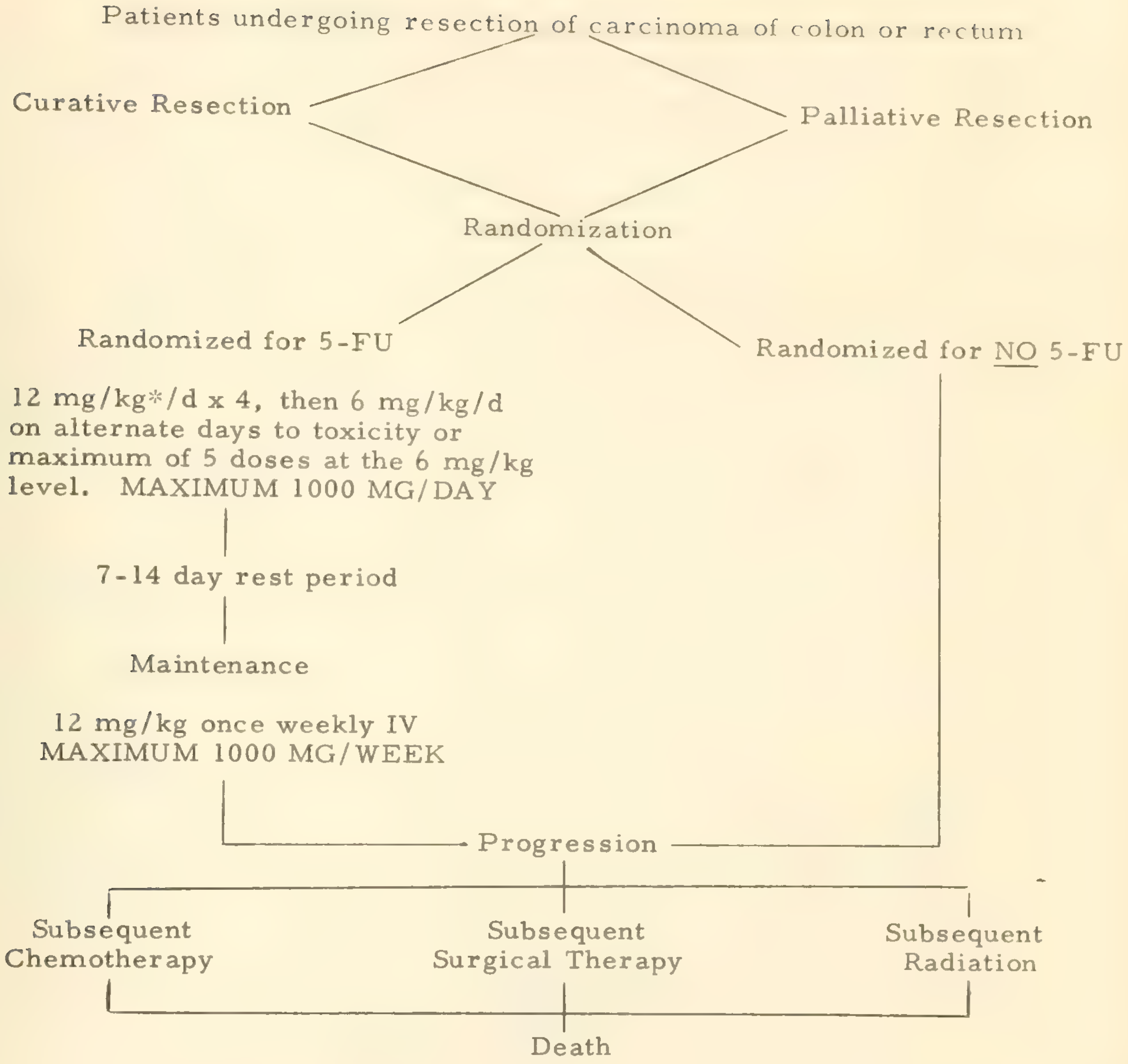
Patient Selection:

1. Resection for primary adenocarcinoma of the colon or rectum.
2. Resected specimen indicated poor prognosis for cure.
3. Patient's clinical condition allows drug therapy.
4. Randomization series determined by clinical impression for residual disease and by microscopic findings.
    a. Clinically curative and no microscopic evidence of residual disease.
    b. Microscopic evidence of residual disease.
5. Patient randomized and treatment started between 14th and 30th PO days.
    a. No drug.
    b. 5-FU in repeated courses.

Drug dosage:

1. IV, 12 mg/kg/d x 5 (Maximum single dose = 1 gm).
2. Drug courses given at six-eight week intervals.
3. Drug courses continued until the 19th post-operative month.

Figure 9. VA Surgical Adjuvant Chemotherapy Study Group Protocol 23.


Patients undergoing resection of carcinoma of colon or rectum
Curative Resection
Palliative Resection
Randomization
Randomized for 5-FU
Randomized for NO 5-FU
12 mg/kg*/d x 4, then 6 mg/kg/d on alternate days to toxicity or maximum of 5 doses at the 6 mg/kg level. MAXIMUM 1000 MG/DAY
7-14 day rest period
Maintenance
12 mg/kg once weekly IV MAXIMUM 1000 MG/WEEK
Progression
Subsequent Chemotherapy
Subsequent Surgical Therapy
Subsequent Radiation
Death

* Based on the lesser of actual and ideal weights.

Figure 10. COG 7041: 5-FU as adjuvant therapy for colo-rectal carcinomas.

INVESTIGATIONAL DRUG BRANCH

In the past fiscal year, the Investigational Drug Branch was established with responsibility for monitoring and coordinating clinical trials on new anticancer drugs sponsored by the Division of Cancer Treatment, liaison with the Food and Drug Administration, and distribution of investigational drugs throughout the United States and the world.

Administratively, the branch consists of the Office of the Chief and the Drug Liaison and Distribution Section.

The Office of the Chief is responsible for (a) monitoring investigational drug evaluation in Phase I, II, and III studies, either as single agents or in combination, (b) liaison with cooperative groups, (c) evaluation and approval of investigatonal drug protocols submitted by investigators conducting independent studies, (d) training of staff associates in drug development, clinical pharmacology, and evaluation of clinical studies of investigational anticancer drugs, and (e) coordination of all branch functions and feedback to the DCT.

These functions are accomplished by the Acting Chief with the aid of two associates, Drs. Handelsman and Wasserman, who are assistants to the Associate Director, CTEP, but are rotating through the Investigational Drug Branch in the first year of their training period.

The following is the detailed report on major accomplishments in the following activities.

SINGLE AGENTS

Evaluation of investigational drugs recently in Phase I, Phase II, and Phase III studies.

1. Phase I Studies: Fifteen investigational drugs, sponsored by the DCT were studied in Phase I as listed below:

Yoshi 864 (NSC 102627)
d-Tetrandrine (NSC 77037)
Thalicarpine (NSC 68075)
Cyclocytidine (NSC 145668)
α-TGdR (NSC 71851)
Soluble Baker's Antifol (NSC 139105)
Cytembena (NSC 104801)
Crystalline Antibiotic (NSC 135758)
Diglycoaldehyde (NSC 118994)
Tritylcysteine (NSC 83265)
Asaley (NSC 167780)
Ftorafur (NSC 148958)
Phosphoramide (69945)
MER (NSC 143769)
BCG (NSC B116328)

The most important results are summarized in Tables 1-11. Phase I studies are accomplished and coordinated through the Phase I Working Group, where the Acting Chief of the Investigational Drug Branch serves as executive secretary. This group provides good communication and coordination between both areas.

2. Phase II Studies: Among the drugs evaluated in Phase II studies summarized in Tables 12-18 were:

CCNU (NSC 79037)
Methyl CCNU (NSC 95441)
Streptozotocin (NSC 85998)
Isophosphamide (NSC 109724)
Cis platinum-diamminedichloride (NSC 119875)
5-Azacytidine (NSC 102816)
Dibromodulcitol (NSC 104800)
ICRF 159 (NSC 129943)

The drugs with established activity where a New Drug Application has been either filed or is being considered include:

Adriamycin (NSC 123127)
BCNU (NSC 409962)
CCNU (NSC 79037)
DTIC (NSC 45388)
Mitomycin C (NSC 26988)
Hexamethylmelamine (NSC 13875)
Bleomycin (NSC 125066)

Within these activities, a detailed comparative review and analysis of three nitrosoureas was conducted by Dr. Wasserman. This included a review of all the known single agent data on CCNU and Methyl CCNU, made to revise clinical brochures on these agents, and the data from Dr. Carter's previous review of BCNU and/or the Bristol N. D. A. for BCNU.

Figure 1 compares the animal tumor data on the nitrosoureas. Figure 2 outlines the sources of data available for clinical comparison of the nitrosoureas. The first three sources were used in this analysis. The data from Sources #1 and #2 are included in the summary data (Source #3).

Figure 3 outlines the methods used for the analysis. All of the data were checked for statistical significance and, when meaningful, are presented with the data. The order of presentation in Figures 4-40 is by degree of activity in the different tumor types. The figures entitled "Brain Tumors--All Types" include the data given specifically for gliomas in separate figures.

Figure 41 summarizes the source of data used for the comparison. There have been, or are now ongoing, only eight direct comparative trials. Only one direct comparative trial has been done between BCNU and CCNU. The other seven trials are between CCNU and MeCCNU. Only two direct comparative trials have shown any significant difference, both by the ALGB in Hodgkin's disease. One trial showed CCNU to be better than BCNU and another study showed CCNU to be better than MeCCNU.

Of the 17 sequential trials, only 2 have shown any significant difference. The data in Hodgkin's disease by the SECG shows BCNU to be better than MeCCNU.

The SWOG data in breast cancer shows BCNU to be better than MeCCNU.

Of the summary data, only in breast and Hodgkin's disease is there any significant difference, but the summary data include the data of the specific trials that also showed the same differences.

COMBINATION CHEMOTHERAPY

Since the major trend in cancer chemotherapy is toward drug combination, the Branch is also monitoring Phase I, II, and III studies on combination chemotherapy. As an example, adriamycin is integrated in 2-, 3-, and 4-drug combinations as shown in Tables 19-21.

LIAISON WITH COOPERATIVE GROUPS

Within these responsibilities, CTEP staff members attended more than 30 Cooperative Group meetings in the past year. This direct communication proved to be important and beneficial for both sides. The CTEP information, both drug- and disease-oriented, is actively required by members of most of the groups. On the other hand, the flow of information from the cooperative groups to the Investigational Drug Branch is smoother and is not delayed until receipt of the minutes of group meetings.

EVALUATION OF INDEPENDENT INVESTIGATOR PROTOCOLS

Within the last year the number of protocols using investigational drugs submitted by independent investigators for approval rapidly increased. These reached an average of 15-20 protocols weekly. This represents a substantial work load, as well as a moral and legal responsibility, for the CTEP staff members.

To meet these requirements the Protocol Review Committee was established to evaluate all protocols submitted by independent investigators. The committee is chaired by the Acting Chief; it meets weekly and evaluates each protocol based on scientific merit and indication for the particular disease. To accomplish this, the guidelines for investigators conducting independent studies have been revised. Reports on the committee meetings are sent to the Associate Director for CTE and complicated protocols are discussed directly with him. If the current rate continues, about 800-1000 protocols of independent investigators may be expected to be submitted to the Branch each year.

HONORS AND AWARDS

1. Slavik, M.: Elected to membership in the American Society for Pharmacology and Experimental Therapeutics.
2. Slavik, M.: Elected to membership in the American Society for Clinical Pharmacology and Therapeutics.
3. Slavik, M.: Elected to membership in the American Association for Cancer Research.
4. Slavik, M.: Elected to membership in the American Society for Clinical Oncology.

5. Slavik, M.: Elected to membership in the American Society for Clinical Toxicology.

6. Slavik, M.: Founding member of the International Associaton for the Study of Lung Cancer.

7. Slavik, M.: Appointed as consulting faculty member of Institute of Clinical Toxicology, Houston, Texas.

8. Penta, J.S.: Special recognition award from NIH for sustained high quality work performance.

9. Penta, J.S.: Section Head chosed as one of 50 nation-wide participants in a Clinical Pharmacology Workshop at the University of California Medical School, San Francisco, California

10. Penta, J.S.: Section Head elected to the Academy of Sciences of the American Pharmaceutical Association, Divison of Pharmacology-Toxicology.

PUBLICATIONS

1. Slavik, M. and Carter, S.K: Bronchogenic carcinoma -- New drugs available for study. Cancer Chemother. Rep. 4:265-269, 1973.

2. Slavik, M., Danilson, D., Keiser, H., and Kenkin, R.: Alterations in metabolism of copper and zinc after administration of 6-azauridine triacetate. Biochem. Pharmacol. 22:2349, 1973.

3. Slavik, M., Lovenberg, W., and Keiser, H.R.: Changes in serum and urine amino acids in pateitns with progressive systemic sclerosis treated with 6-azauridine triacetate. Biochem. Pharmacol. 22:1295-1300, 1973.

PRESENTATIONS

1. Slavik, M.: Analysis of Side Effects of Anticancer Drugs in Man. NCI-VA Medical Oncology Branch, VA Hospital, Washington, D.C., May 3, 1973.

2. Slavik, M.: If Drug Fails in Phase I Clinical Trial, Should There be a Pharmacological Effort to Investigate Why a Promising Antitumor Agent in Animals Fails in Man? First Annual Experimental Therapeutics Pharmacology Workshop, Brook Lodge, May 16-17, 1973.

3. Slavik, M.: Critical Variables in Phase I Study Design. Phase I Working Group Meeting, NCI, Bethesda, June 14, 1973.

4. Slavik, M.: New Drugs Available for Study: Crystalline Antibiotic (NSC 135753) and Diglycoaldehyde (NSC 118994). Phase I Working Group Meeting, NCI, Bethesda, June 14, 1973.

5. Slavik, M.: Clinical Potential of Tetrahydrouridine. Tetrahydrouridine Review Meeting, NCI, Bethesda, August 2, 1973.

6. Slavik, M.: An Update on Clinical Studies with Chromomycin A_3 and 5-Azacytidine. Western Cooperative Group Meeting, San Francisco, September 14, 1973.

7. Slavik, M.: New Drugs Available for Study - Methyl CCNU - Parenteral Form and Fluorodopan. Phase I Working Group Meeting, NCI, Bethesda, October 11, 1973.

8. Slavik, M.: Investigational Drugs Entering Phase I Study: Diglycoaldehyde, Crystalline Antibiotic, and Tritylcysteine. New Drug Liaison Meeting, NCI, Bethesda, December 11, 1973.

9. Slavik, M.: Single Agents and Combination Chemother of the Ovarian and Cervical Cancer. Gynecologic Group Meeting, Phoenix, December 14, 1973

10. Slavik, M.: 1,3,4-Thiadiazol-2-Amino (NSC 4728) - A New Drug Available for Clinical Studies. Phase I Working Group Meeting, NCI, Bethesda, January 9, 1974.

11. Slavik, M.: Some Current Phase I Approaches with Combinations Involving Adriamycin. Phase I Working Group Meeting, NCI, Bethesda, January 9, 1973.

12. Slavik, M.: Update on Phase I Clinical Studies On New Investigational Drugs. New Agents Committee at Central Oncology Group Meeting, Park City, Utah, January, 10, 1974.

13. Slavik, M.: Critical Variables in Phase I Studies. Roswell Park Memorial Institute, Buffalo, January 25, 1974.

14. Slavik, M.: New Investigational Drugs Recently in Phase I Clinical Studies. Southwest Oncology Group Meeting, Tucson, February 13, 1974.

15. Slavik, M.: Effect of Some Investigational Drugs on Aminio Acid Metabolism. Tulane University, New Orleans, February 25, 1974.

16. Slavik, M. and Wasserman, T.H.: Overview of Clinical Studies on Adriamycin Combinations. Combination Chemotherapy Subcommittee Meeting, NCI, Bethesda, March 21, 1974.

17. Slavik, M. and Wasserman, T.H.: Overview of Clinical Studies on Adriamycin and Cytoxan Combination. Combination Chemotherapy Subcommittee Meeting, NCI, Bethesda, March 21, 1974.

18. Penta, J.S.: S-Trityl-L-Cysteine -A New Anticancer Drug. Phase I Working Party, NCI, Bethesda, June 14, 1973.

19. Penta, J.S.: Antitumor Activity, Pharmacology, and Preclinical Toxicology of Three New Anticancer Drugs: (a) S-Trityl-L-Cysteine, (b) Gallium Nitrate, (c) d-Tetrandrine. Southwest Oncology Group, San Antonio, October 9-12, 1973.

0. Penta, J.S.: Pharmacological Response to Combination Cancer Chemotherapeutic Agents. Department of Oncology, School of Medicine, Wayne State University, Detroit, December 12, 1973.

1. Penta, J.S.: Investigational New Anticancer Drugs and FDA Guidelines. Detroit Cancer Society, Detroit, December 11, 1973.

2. Penta, J.S.: *C. parvum*. A new Immunoadjuvant for Use in Human Malignancy. Phase I Working Group, NCI, Bethesda, January 9, 1974.

3. Penta, J.S.: Triple-Drug Combination of Current Interest in the Treatment of Human Breast Cancer. Cooperative Breast Group Meeting, New Orleans, January 23, 1974.

24. Penta, J.S.: A Summary Comparison between Tice BCG and Pasteur BCG. Eastern Cooperative Oncology Group Immunotherapy Subcommittee, Miami, February 10-12, 1974.

Table 1. Phase I studies with Yoshi 864 (Altman, COG #7111).*

# Pts. Evaluated	Dose Schedule	Dose mg/m^2			Toxicity (%)		
		Starting	Highest Escalated(HED)	Maximum Tolerated	GI	Hem.	CNS Somnolence**
26	Daily x 5 IV push	0.25mg/kg (9.25)	2.7 mg/kg (81.4)	2.0	18	52 Leukopenia + thrombocytopenia when dose 1.5mg/kg	100% HED

* Includes pharmacokinetic studies. Responses seen in CML and Head and Neck.

** Dose limiting toxicity.

Table 2. Phase I studies with d-tetrandrine (DeConti, ECOG #1773).

# Pts. Evaluated	Dose Schedule	Dose mg/m^2			Toxicity
		Starting	Highest Escalated	Maximum Tolerated	
25	Single IV infusion	50	540		Bradycardia + cardiovascular collapse after rapid IV push but not after 1 hr infusion. Local reaction at the site of injection.
	Daily x 5 IV infusion	50	270		

Table 3. Studies with asaley (Freireich).

# Pts. Evaluated	Dose Schedule	Dose mg/m^2			Toxicity (%)		
		Starting	Highest Escalated	Maximum Tolerated	GI	Hem.	CNS
20	d x 4 q 3-5 wks	200	1000	800	25	80	8

Table 4. Phase I Studies with thalicarpine (Creaven).*

# Pts. Evaluated	Dose Schedule	Dose mg/m^2 Starting	Dose mg/m^2 Highest Escalated (HED)	Dose mg/m^2 Maximum Tolerated	Toxicity GI	Toxicity Cardio	Toxicity CNS**
30	Single IV 1 hr infusion 500ml D5/W	100	1900	1400		+	100% HED

* Pharmacokinetic studies included ** Dose limiting toxicity

Table 5. Phase I studies with cyclocytidine.

Investigator	# Pts. Evaluated	Dose Schedule	Dose mg/m^2 Start	Dose mg/m^2 Highest Escalated (HED)	Dose mg/m^2 Maximum Tolerated	Toxicity (%) GI	Toxicity (%) Hem.	Toxicity (%) Jaw Pain	Toxicity (%) Postural Hypotension*
Freireich**	20	d x 5 d x 10	100 75	675	450		50	92%>200mg/m^2	50% HED
Frei***	19	d x 5 d x 10	100 100	600		30	53	100%>200 mg/m^2	76% in adults > 150 mg/m^2
Krakoff	6	d x 7	100	500		+	+	+	0

* Dose limiting toxicity ** Responses melanoma 2/7 *** 1 Response ALL (Child)

Table 6. Studies with α-TGdR (Freireich & Khaliq).

# Pts. Evaluated	Dose Schedule	Dose mg/m^2 Starting	Dose mg/m^2 Highest Escalated	Dose mg/m^2 Maximum Tolerated	Toxicity
12	d x 5	150	600		No toxicity (no activity)

Table 7. Phase I studies with soluble Baker's antifol.

			Dose mg/m^2			Toxicity		
Investigator	# Pts. Evaluated	Dose Schedule	Starting	Highest Escalated	Maximum Tolerated	GI	Hem.	Skin
Freireich	11	d x 5	10	100			+	+
Bertino	10	d x 5	10	40				+
	11	Single dose IV	20	300		+	+	

Table 8. Phase I Studies with Cytembena.

			Dose mg/m^2			Toxicity			
Investigator	# Pts. Evaluated	Dose Schedule	Start	Highest Escalated (HED)	Maximum Tolerated	GI	Hem.	Autonomic Discharge**	Renal**
Moertel*	16	d x 5	50	470	300	100%		100% HED	> 300mg/m^2
Henderson	14	d x 20	25	125		MTD not yet reached. No toxicity or activity yet observed.			

* Pharmacokinetic studies included

** Dose limiting toxicity

Table 9. Phase I Studies with tritylcysteine (Frei).

		Dose mg/m^2			Toxicity		
# Pts. Evaluated	Dose Schedule	Starting	Highest Escalated	Maximum Tolerated	GI	Hem.	Hepato.
12	d x 5 IV	4.0	100		No toxicity or activity		

Table 10. Phase I studies with piperazinedione.

Investigator	# Pts. Evaluated	Dose Schedule	Dose mg/m^2			Toxicity		
			Starting	Highest Escalated	Maximum Tolerated	GI	Hem.	Hepato.
Freireich	8	Single IV	1.5	18.0		1 pt. hematologic toxicity 1 PR (?) renal cell		
Henderson*	8	Weekly IV	0.3	1.5		+	+	
Krakoff	12	d x 5 IV	0.3	2.0		No toxicity, no activity yet		
Pratt	1	d x 5 IV (children)	0.3					

* No activity

Table 11. Phase I studies with diglycoaldehyde.

Investigator	# Pts. Evaluated	Dose Schedule	Dose mg/m^2			Toxicity		
			Starting	Highest Escalated	Maximum Tolerated	GI	Hem.	Renal*
Freireich	6	d x 5	30	600		No activity or toxicity		
Karon**	6	d x 5	30	2000	1500	+		+
Mittelman***	12	d x 5	30	4000	2000	+		+
Bleyer****	1	d x 5	1000					

* Dose limiting toxicity

** 1 Response in child with ALL

*** Responses in renal cell and mesothelioma

**** Pharmacokinetic studies included

Table 12. Summary Phase II studies with CCNU.

Tumor Type	# Pts. Evaluable	# CR	# CR+PR	% Response
Breast Carcinoma	171	1	20	11.6
Colon Carcinoma	222	3	20	9.0
Bronchogenic Carcinoma	262	8	46	17.5
Pancreatic Carcinoma	19	0	3	15.8
Ovarian Carcinoma	22	2	7	31.8
Malignant Melanoma	126	1	19	15.0
AML	2	0	0	0
ALL	21	0	0	0
Hodgkin's Disease	57	7	26	45.6
Non-Hodgkin's Lymphomas	36	4	10	27.8
Malignant Gliomas	73	1	27	36.9
ALL CNS Tumors	115	1	47	40.9
Cervix	5	0	1	20.0
CLL	2	1	0	"50"
CML	0	0	0	0
Esophagus	19	1	2	10.5
Myeloma	3	0	1	33.3
Head and Neck	50	0	4	8.0
Renal Cell	59	0	4	6.8
Sarcoma	22	1	1	4.5
Skin	29	0	5	17.2
Gastric	35	1	1	2.9
Total	1277	32	244	18.8

Table 13. Phase II studies with isophosphamide.

Tumor Type	# Pts. Evaluated	# CR	# CR+PR	% Response	Investigator
Breast Carcinoma	20*		4	20	Ahman, 1973
Colon Carcinoma	33**		1	3	Kovach, 1973
Myeloma	30***		0	0	Salmon, 1973

*4000mg/m^2 IV q 4wks **4000mg/m^2 IV q 3wks ***3000-5000mg/m^2 IV q 3wks

Table 14. Summary Phase II studies with MeCCNU.

Tumor Type	# Pts. Evaluable	# CR	# CR+PR	% Response
Breast Carcinoma	95	0	3	3
Colorectal Carcinoma	148	4	18	12
Lung Cancer	295	4	30	10
Pancreatic Carcinoma	13	0	0	0
Ovarian Carcinoma	16	0	0	0
Malignant Melanoma	124	2	17	14
AML and ALL	20	0	2	10
Hodgkin's Disease	66	2	17	26
Non-Hodgkin's Lymphomas	44	4	9	20
CNS Tumors	46	1	17	37
Sarcoma	76	0	5	7
Head and Neck	51	0	8	16
Cervical	24	0	4	17
Gastric	28	0	1	4
Renal	21	0	0	0
Prostatic	19	1	1	11
Testes	4	0	0	--
Total	1090	18	132	12.1

Table 15. Phase II studies with streptozotocin.

Tumor Type	# Pts. Evaluable	# CR	# CR+PR	% Response
Breast Carcinoma	17	0	2	11.7
Colon Carcinoma	65	0	6	9.2
Bronchogenic Carcinoma	5	0	2	"40"
Pancreatic Carcinoma	27	0	3	11.1
Ovarian Carcinoma	9	0	0	0
Malignant Melanoma	19		2	10.5
AML	2	0	0	0
ALL	5		1	20
Malignant Lymphoma	40		11	27.5
Islet Cell	90	15	50	55.5
Carcinoid	36		7	19.4
Sarcomas	15		2	13.3
Head and Neck	7		2	11.8
Total	337	15	88	26.1

Table 16. Phase II studies with cis-platinum (II) diamminedichloride.

Tumor Type	# Pts. Evaluable	# CR	# CR+PR	% Response
Breast Carcinoma	9	0	2	22.2
Colon Carcinoma	52	0	0	0
Bronchogenic Carcinoma	16	0	1	6.25
Pancreatic Carcinoma	2	0	0	0
Ovarian Carcinoma	21	0	4	19
Malignant Melanoma	11		1	9.0
AML	19	1	2	10.5
ALL	2	0	0	0
Malignant Lymphomas	15	0	7	46.6
Sarcomas	24		8	33.3
Testicular Tumors	15	3	9	60.0
Head and Neck	16	0	2	12.5
Prostate	5	0	0	0
Bladder	1		1	
Total	208	4	37	17.8

Table 17. Phase II studies with 5-azacytidine.

Tumor Type	# Pts. Evaluable	# CR	# CR+PR	% Response
Breast Carcinoma	53	0	11	20.7
Colon Carcinoma	52	0	5	9.6
Bronchogenic Carcinoma	9	0	0	0
Pancreatic Carcinoma	2	0	1	
Ovarian Carcinoma	4	0	1	25
Malignant Melanoma	23	0	4	17.4
AML	53	11	20	37.7
ALL	32	2	3	9.3
CML	7	1	1	14.3
Malignant Lymphoma	2	0	1	
Malignant Gliomas	1	0	1	
Head and Neck	5		1	20.0
Sarcomas	4	0	0	0
Total	247	14	49	19.8

Table 18. Phase II studies with dibromodulcitol.

Tumor Type	# Pts. Evaluable	# CR	# CR+PR	% Response
Breast Carcinoma	53	1	20	37.7
Colon Carcinoma	42	1	4	9.5
Bronchogenic Carcinoma	255	0	37	14.5
Ovarian Carcinoma	8	0	1	12.5
Malignant Melanoma	42	1	8	19.0
AML	2	0	1	"50"
ALL	15	0	2	15
CML	8	8		"100"
Malignant Lymphoma	19	2	9	47.4
Head and Neck	50	2	11	
Sarcoma	44		7	16
Renal Cell	19		4	21.0
Mesothelioma	3		1	"33"
Prostate	3		1	"33"
Total	563	15	106	18.8

Table 19. Adriamycin 2-drug combinations.

Adriamycin + Cytoxan
Adriamycin + 5-FU
Adriamycin + Methotrexate
Adriamycin + Vincristine
Adriamycin + Bleomycin
Adriamycin + CCNU
Adriamycin + Methyl CCNU
Adriamycin + ICRF-159
Adriamycin + DTIC
Adriamycin + Dibromodulcitol
Adriamycin + 5-Azacytidine
Adriamycin + Streptozotocin
Adriamycin + Platinum
Adriamycin + Prednisone

Table 20. Adriamycin 3-drug combinations.

Adriamycin + Cytoxan + 5-FU
Adriamycin + Cytoxan + Methotrexate
Adriamycin + Cytoxan + Methyl CCNU
Adriamycin + Cytoxan + Prednisone
Adriamycin + Cytoxan + Vincristine
Adriamycin + Methotrexate + Vincristine
Adriamycin + DTIC + Vincristine
Adriamycin + MeCCNU + Vincristine
Adriamycin + Prednisone + Vincristine
Adriamycin + Prednisone + Melphalan
Adriamycin + DTIC + VM 26
Adriamycin + Bleomycin + Hexamethylmelamine
Adriamycin + Bleomycin + 5-FU

Table 21. Adriamycin 4-drug combinations.

Adriamycin + Cytoxan + Vincristine + DTIC
Adriamycin + Cytoxan + Vincristine + Actinomycin D
Adriamycin + Cytoxan + Vincristine + Bleomycin
Adriamycin + Cytoxan + Vincristine + Prednisone
Adriamycin + Cytoxan + Vincristine + Melphalan
Adriamycin + Cytoxan + 5-FU + Methotrexate
Adriamycin + Ara-C + Vincristine + Prednisone
Adriamycin + 6-MP + Vincristine + Prednisone
Adriamycin + DTIC + Velban + Bleomycin

IP L1210 - all three agents highly active
IC L1210 - CCNU > MeCCNU = BCNU
B16 melanoma - MeCCNU > CCNU = BCNU
Lewis lung - Meccnu ≫ CCNU > BCNU

Figure 1. Animal tumor data - comparison of nitrosoureas

1. Direct, randomized, comparative trials in a given tumor type
2. Sequential trials in a given tumor type by a given group or investigator
3. Summarized Phase II-III data in a given tumor type.
4. Clinician's impressions
5. Combinations of the above

Figure 2. Sources of data for clinical comparison of the nitrosoureas

1. Review of all major studies using a nitrosourea.
2. "Evaluable" patients include those who received a nitrosourea on an appropriate dose schedule and who could be evaluated for objective response.
3. A "response" is a CR or PR of greater than or equal to a 50% tumor shrinkage for at least 1 month.
4. Response rate = $\frac{\text{No. responding patients}}{\text{No. evaluable patients}}$

Figure 3. Methods used in analysis

BTSG - 72-01

XRT + BCNU vs XRT + MeCCNU
No data as yet

Figure 4. Gliomas: Direct, randomized comparative clinical trial with the nitrosoureas (from Group minutes, Oct. 1973).

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	27	11	41
CCNU (2)	16	6	38
MeCCNU (3)	27	8	30

Figure 5. Brain tumors -- all types. Sequential trials at BCRC. (1) Walker, CCR, 1970, (2) Rosenblum, Neurosurgery, 1973, (3) Kaplan, New Drug Liaison Mtg, Feb. 1973.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	94	44	47
CCNU (2)	21	7	33
MeCCNU			

Figure 6. Brain tumor--all types. Sequential trials at U. Calif. at San Francisco. (1) Fewer, JAMA, 1972, Wilson, CCR, 1970, (2) Fewer, CCR, 1972.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU			
CCNU (1)	30	10	33
MeCCNU (2)	21	7	33

Figure 7. Brain tumors -- all types. Sequential trials by SWOG. (1) Hoogstraten, Cancer, 1973, (2) SWOG minutes, Oct. 1973.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	20	10	50
CCNU (2)	11	4	36
MeCCNU (3)	20	6	30

Figure 8. Gliomas--sequential trials at BCRC. (1) Walker, CCR, 1970, (2) Rosenblum, Neurosourgery, 1973, (3) Kaplan, New Drug Liaison Mtg, Feb. 1973.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	48	23	48
CCNU (2)	15	5	33
MeCCNU			

Figure 9. Gliomas--sequential trials at U. of California, San Francisco. (1) Fewer, JAMA, 1972, Wilson, CCR, 1970 , (2) Fewer, CCR, 1972.

	No. Pts. Evaluable	No. Pts. Responding	% Response	
BCNU (1)	142	67	47	
CCNU (2)	115	47	41	p = .078
MeCCNU (2)	48	15	31	

Figure 10. Brain tumors (all types). Summarized Phase II-III data. (1) Agre, Bristol, N. D. A., (2) Wasserman, CTEP review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	85	38	45
CCNU (2)	73	27	37
MeCCNU (2)	20	6	30

Figure 11. Gliomas - Summarized Phase II-III data. (1) Agre, Bristol, N. D. A., (2) Wasserman, CTEP review.

	No. Pts. Evaluable	No. Pts. Responding	% Response		Mean duration
BCNU	28	10	36	p = .01	23 days
CCNU	26	18	69		71 days

Figure 12. Hodgkin's disease--Direct, randomized comparative clinical trial with the nitrosoureas (ALGB 6953, BCNU vs CCNU, from Carter, CCR, May 1973). Patients were failures on combination therapy. The toxicity from CCNU was slightly greater than from BCNU and slightly fewer patients receiving CCNU had prior combination chemotherapy than those patients receiving BCNU (80.9% vs 86.8%).

	No. Pts. Evaluable	No. Pts. Responding	% Response	
Hodgkin's				
CCNU	27	14	52	p = 0.56
MeCCNU	19	5	26	
Non-Hodgkin's lymphomas				
CCNU	16	3	19	
MeCCNU	18	3	17	

Figure 13. Hodgkin's disease + other lymphomas. Direct, randomized comparative clinical trial with the nitrosoureas (CCNU vs MeCCNU, ALGB 7252, from Group minutes, Nov. 1973). Patients have advanced disease with much prior therapy. Study closed.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	29	10	34
CCNU (2)	25	7	28
MeCCNU			

Figure 14. Hodgkin's disease. Sequential trials by the WOG. (1) WOG minutes, May 1972, (2) Group minutes, Sept. 1973.

	No. Pts. Evaluable	No. Pts. Responding	% Response	
BCNU (1)	63	25	40	
CCNU				p = .018
MeCCNU (2)	17	1	6	

Figure 15. Hodgkin's disease. Sequential trials by SEOG. (1) Lessner, *Cancer*, 1968, and Group minutes, 1970, (2) Group minutes, Nov. 1970.

	No. Pts. Evaluable	No. Pts. Responding	% Response	
BCNU (1)	213	94	44	
CCNU (2)	84	40	48	p = 0.47
MeCCNU (2)	52	15	29	

Figure 16. Hodgkin's disease. Summarized Phase II-III data. (1) Agre, Bristol N. D. A (2) Wasserman, CTEP review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	21	4	19
CCNU			
MeCCNU (2)	21	3	14

Figure 17. Non-Hodgkin's lymphomas-Sequential trials by the SEOG. (1) Lessner, *Cancer*, 1968, and Group minutes, 1970 (20 Group minutes, Nov 1973.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	107	30	28
CCNU (2)	52	13	25
MeCCNU (2)	42	6	14

igure 18. Non-Hodgkin's lymphomas. Summarized Phase II-III data. (1) Carter, *Adv in Cancer Res*, 1972 (2) Wasserman, CTEP review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU			
CCNU (1)	19	1	5
MeCCNU (2)	19	5	26

igure 19. Melanoma - Sequential trials at Mayo Clinic (1) Ahmann, *Cancer Res*, 1972 (2) Ahmann, personal communication.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	98	19	19
CCNU (2)	27	7	26
MeCCNU			

Figure 20. Melanoma - Sequential trials by COG. (1) Ramirez, *CCR*, 1972 (2) Wasserman, CTEP review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	123	22	18
CCNU (2)	126	19	15
MeCCNU (2)	62	11	18

Figure 21. Melanoma - Summarized Phase II-III data. (1) Luce, *Cancer*, Dec. 1972 (2) Wasserman, CTEP review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU	64	8	12.5
CCNU	75	7	9
MeCCNU	40	7	17.5

Figure 22. Colon cancer. Sequential trials at Mayo Clinic (Moertel, CCR, May, 1973.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU			
CCNU (1)	31	0	0
MeCCNU (2)	57	3	5

Figure 23. Colon cancer. Sequential trials by theSWOG. (1) Hoogstraten, Cancer, 1973 (2) Group minutes, Oct. 1973.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	128	17	13
CCNU (2)	222	20	9
MeCCNU (2)	125	14	11

Figure 24. Colon cancer. Summarized Phase II-III data. (1) Carter, Adv in Cancer Res, 1972 (2) Wasserman, CTEP review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
CCNU (1)	46	2	4
MeCCNU (1)	47	4	9
BCNU (2)	25	3	12

Figure 25. Lung cancer. Direct, randomized comparative clinical trial with the nitrosoureas. (1) Carr, report, Nov. 1973 (2) Ahmann, CCR, June 1972.

	No. Pts. Evaluable	% Response	Survival
CCNU	102-113	STILL CODED	
MeCCNU	102-113		

Figure 26. Lung cancer. Direct, randomized comparative clinical trial with the nitrosoureas. (VA Lung protocol 13, from Group minutes, Oct. 1973).

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU			
CCNU (1)	45	8	18
MeCCNU (2)	112	18	16

Figure 27. Lung cancer. Sequential trials by the SWOG. (1) Hoogstraten, *Cancer*, 1973 (2) Group minutes, Oct. 1973.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	19	4	21
CCNU (2)	32	8	25
MeCCNU			

Figure 28. Lung cancer. Sequential trials by the C OG. (1) Ramirez, *CCR*, 1972 (2) Wasserman, CTEB review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	83	9	11
CCNU (2)	262	46	18
MeCCNU (2)	195	24	12

Figure 29. Lung cancer. Summarized Phase II-III data. (1) Carter, *Adv in Cancer Res*, 1972 (2) Wasserman, CTEP review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	38	1	3
CCNU (2)	56	8	14
MeCCNU (2)	65	10	15

Figure 30. Lung cancer (squamous cell). Summarized Phase II-III data. (1) Selawry, CCR, March 1973 (2) Wasserman, review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	14	0	0
CCNU (2)	54	9	17
MeCCNU (2)	40	2	5

Figure 31. Lung cancer (adenocarcinoma). Summarized Phase II-III data. (1) Selawry, CCR, March 1973 (2) Wasserman, review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	15	3	20
CCNU (2)	48	7	15
MeCCNU (2)	34	8	24

Figure 32. Lung cancer (small cell). Summarized Phase II-III data. (1) Selawry, CCR, March 1973 (2) Wasserman, review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	15	1	7
CCNU (2)	37	9	24
MeCCNU (2)	7	1	14

Figure 33. Lung cancer (large cell). Summarized Phase II-III data. (1) Selawry, CCR, March 1973 (2) Wasserman, review.

	No. Pts. Evaluable	No. Pts. Responding	% Response	Duration
CCNU	29	4	14	3 mo.
MeCCNU	31	1	3	1 1/2 mo.

Figure 34. Breast cancer. Direct, randomized comparative clinical trial with the nitrosoureas (SWG 449, Group minutes, May 1973).

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	16	0	0
CCNU (2)	21	2	10
MeCCNU (3)	22	1	5

Figure 35. Breast cancer. Sequential trials at Mayo Clinic. (1) Ahmann, *CCR*, 1972 (2) Ahmann, *Cancer Res*, 1973 (3) *ASCO* Abstract, 1973.

	No. Pts. Evaluable	No. Pts. Responding	% Response	
BCNU (1)	36	13	36	
CCNU (2)	36	7	19	p = .016
MeCCNU (3)	17	1	6	

Figure 36. Breast cancer. Sequential trials by SWOG. (1) Bonnet, *CCR*, 1973 (2) Hoogstraten, *Cancer*, 1973 (3) Group minutes, Oct 1973.

	No. Pts. Evaluable	No. Pts. Responding	% Response	
BCNU (1)	76	16	21	p=.083
CCNU (2)	171	20	12	p = <. 01
MeCCNU (3)	85	3	4	p=,054

Figure 37. Breast cancer. Summarized PhaseII-III data. (1) Carter, *Adv in Cancer Res*, 1972 (2) Wasserman, CTEP review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	11	3	27
CCNU (2)	27	3	11
MeCCNU			

Figure 38. Head & Neck. Sequential trials by COG. (1) Ramirez, CCR, 1972 (2) Wasserman, CTEP review.

	No. Pts. Evaluable	No. Pts. Responding	% Response
BCNU (1)	25	4	16
CCNU (2)	50	4	8
MeCCNU (2)	31	3	10

Figure 39. Head & Neck. Summarized Phase II-III data. (1) Carter, Adv in Cancer Res, 1972 (2) Wasserman, CTEP review

	No. Pts. Evaluable	No. Pts. Responding	% Response	
CCNU	137	2	1	
MeCCNU	134	8	6	
		GI CANCER		
CCNU	44	0	0	p=.054
MeCCNU	51	6	12	

Figure 40. Mixed solid tumors. Direct, randomized comparative clinical trial withthe nitrosoureas. Very low overall response rate. Low doses given, with little toxicity. Study closed (ALGB 7282, CCNU vs MeCCNU, Group minutes, Nov. 1973).

Tumor Type	Comparative Trials	No. Sequential Trials	Summary Data (1)
liomas	1	2	+
rain (all types)	No data	3	+
odgkin's disease	2	2	+
on-Hodgkin's lymphmas	1	1	+
Melanoma	-	2	+
Colon cancer	-	2	+
Lung cancer	2	2	+
Breast cancer	1	2	+
Head and neck cancer	-	1	+
Mixed solid tumors	1	-	-
Total	8 (2)	17	

Figure 41. Sources of data for clinical comparison of the nitrosoureas. (1) + = data available (2) 1 study of CCNU vs BCNU; 7 studies of CCNU vs MeCCNU.

DRUG LIAISON AND DISTRIBUTION SECTION

The Section is responsible for compliance with Food and Drug Administration regulations regarding the use of investigational anticancer drugs in man, for supply and distribution of both investigational and commercially available drugs to various clinical investigators throughout the United States and many foreign countries, and for serving as an interface between the various clinical investigators and NCI staff regarding any problems concerning drug stability, purity, and dose formulation.

The Section Head maintains a continuing up-to-date knowledge of the clinical use of all anticancer drugs for which an Investigational New Drug (IND) application is held in order to effectively plan and administer the supply and distribution of clinical drugs for the cooperative groups and independent investigators. Since the Section is also involved in the preclinical aspects of the drugs, the Section Head is a member of the following drug-oriented committees:

1. Drug Status Committee (Chairman)
2. Operating Committee (Executive Secretary)
3. Decision Network Committee (Executive Secretary)
4. Drug Evaluation Committee
5. Quality Control Committee

During the past year the section, for the first time, prepared and submitted an IND application for a vaccine, Bacillus of Calmette-Guerin (BCG). Because FDA regulatory requirements for a biological product differ from that of a drug, it was necessary to become familiar with the clinical and technical aspects of the vaccine in order to prepare the IND application. After a meeting with FDA, the IND application was prepared and submitted.

Also during the past year, IND applications were prepared and submitted for drugs developed in Czechoslovakia, Japan, and the Soviet Union. In the course of preparing the IND applications, it became necessary to meet some of the problems inherent in starting clinical trials with a drug developed in a foreign country. Discussions took place with FDA and, although no official policy was established, some minimal requirements were established and the IND applications for the foreign drugs were successfully submitted.

The Section worked with sponsors of the New Drug Applications (NDA) for DTIC, adriamycin, and Elliott's B solution, which is a vehicle intended for intrathecal administration. This involved collection of clinical data and following up on adverse drug reactions reports. Also, the Section Head advised a number of physicians in private practice with regard to making IND applications for use of investigational anticancer drugs.

The growth of the Cancer Therapy Evaluation Program is reflected in its impact on the Drug Liaison and Distribution Section. The section holds an IND for 119 drugs which comprise about 200 different dose formulations. Seven hundred (700) new investigators registered with the section as recipients of investigational drugs. Nine hundred-fifty (950) protocols were monitored by the section. Nearly 14,000 drug requests were processed during the year and 21 adverse drug reaction reports were sent to the FDA.

A brief description of drugs for which INDs were submitted this year is as follows:

1. Asaley (NSC 167780) is a synthetic derivative of the acetylated form of sarcolysin to which the amino acid L-leucine, has been added. Since the molecular structure of asaley is similar to that of Alkeran®(melphalan), it is thought to be an alkylating agent. Asaley is one of three drugs which the Soviet Union has given to the United States for clinical evaluation. It is reported to have activity in rats with sarcoma 45 and in rats with Walker 256 carcinosarcoma. In the latter system, a single dose of 94 mg/kg, or a daily dose of 40 mg x 10 induced tumor growth inhibition. Asaley has also been administered by the IM and PO route, both as a single dose and as a daily dose, to rats with 7-day old sarcoma 45. A single dose ranging from 24.5 mg/kg to 188 mg/kg showed complete tumor regression in 50-100% of the animals by day 30. However, a dose regimen of 10-40 mg/kg/d x 7-10 produced complete tumor regression in only 13-22% of the animals. It also appeared that leukopenia and immunosuppression were more pronounced with fractional doses than with single doses. Preclinical toxicity data are available only for rats, mice, and rabbits. Toxic manifestations include depression of the WBC, necrosis of gut mucosa, kidney, and liver, and inhibition of weight gain in young rats and mice. Clinically, the Soviets have reported activity in ovarian cancer, breast cancer, malignant melanoma, and myeloma. The drug was introduced into clinical trial on the basis of the Soviet clinical data, and is currently being studied at the M. D. Anderson Hospital and Tumor Institute. The drug was formulated for clinical trials by the Soviets as a 250 mg capsule.

2. Diglycoaldehyde (Inosine Dialdehyde, NSC 118994) Inosine dialdehyde is a synthetic purine which is thought to exert its action through inhibition of DNA, RNA, and protein synthesis. It has also been suggested that it inhibits ribonuclease through peptidide bond formation with lysine residues, thus inhibiting RNA. Feedback inhibition has also been suggested. The drug shows antitumor activity against the spontaneous AKR leukemia, on both a chronic and intermittent schedule, and in the P388 leukemia system and in Lewis lung.

System	Route	Schedule	Dose (mg/kg)	% ILS
AKR	IP	D1-9	25	113
AKR	IP	D1,5,9	240	122
P388	IP	D1-10	140	109
LL	IP	D1-11	150	43

The drug shows negative activity against B16 melanoma, Walker 256, and the rodent ependymoblastoma. Preclinical studies in dogs and monkeys indicate cardiovascular (dog and monkey), respiratory (dog and monkey), liver (monkey), and renal (monkey) toxicity, and pancreatitis (monkey). Little hematopoietic depression was seen at any dose in either the dog or monkey. Toxicology data indicates that rapid administration of the drug should be avoided to prevent hypotension. The drug is formulated for parenteral administration as a 500 mg vial and a 1 gm vial. Phase I clinical trials have started at M. D. Anderson Hospital and Tumor Institute, Roswell Park Memorial Institute, and at Children's Hospital, Los Angeles.

3. Piperazinedione (Crystalline Antibiotic, NSC 135758) This drug is a crystalline antibiotic derived from fermentation products of a Streptomyces strain. It is thought to exert its antitumor effects by inhibition of DNA synthesis in the manner of an alkylating agent. An alkylating agent mechanism of action is suggested because L1210 resistant to cyclophosphamide shows moderate cross-resistance to the antibiotic. The drug shows activity in a number of experimental systems including L1210 leukemia, P388 leukemia, spontaneous AKR leukemia, Walker 256, B16 melanoma, Lewis lung, and the intracranially ependymoblastoma. In this latter system the drug is active when administered either chronically or on an intermittent schedule, as shown below:

Route	Schedule	Dose (mg/kg)	% ILS
IP	D1-9	1.0	172
IP	Q3h, D1, 5, 9	0.75	143
IP	Q3h, D1 only	2.0	77

Against L1210, an optimal dose of 6 mg/kg administered IP on day 1 only shows a % ILS > 401 with 6/10 survivors and 8/8 survivors for normal animals. Against B16 melanoma, an IP dose of 10 mg/kg on day 1 only shows a % ILS of 42, and an IP dose of 2 mg/kg/d x 11 shows a % ILS of 57 against the Lewis lung. Preclinical toxicology studies in large animals (dog and monkey) indicate the following toxicities:

Toxicity	Manifestation
GI	Hemorrhages, necrosis, villous atrophy
Hematologic	Anemia, leukopenia, thrombocytopenia
Lymphoid	Lymphoid depletion
Reproductive system	Testicular degeneration
Respiratory	Congestion, edema, hemorrhages, infection

The drug is formulated for clinical use as a 5 mg vial. Phase I clinical trials are being conducted at Memorial-Sloan Kettering Institute, Roswell Park Memorial Institute, and M. D. Anderson Hospital and Tumor Institute.

4. Citrated Solution of Gallium Nitrate (NSC 15200) Gallium nitrate represents the second drug with a heavy-metal moiety to be introduced into clinical trial as an anticancer agent. The first one was cis-platinum (II)-diamminedichloride. The use of gallium-67 or gallium-72 isotopes has shown a preferential localization in neoplastic tissues of rodents and man. In addition, the increase in tumor uptake has been shown in the C3H/HeJ mammary tumor, P-1798 lymphosarcoma, R-3259 rat sarcoma, and Walker 256. The nitrate salt of gallium has been shown to inhibit the growth of the latter. Also, higher concentrations of gallium-67 were recorded in the thymus and lymph nodes of AKR/J mice with lymphoma than in non-tumor bearing mice. Toxicity in the dog and monkey was primarily hepatic, with renal abnormalities as manifested by proteinurea in the monkey. High doses produced anemia, but not leukopenia. In addition, there is no

difference in canine toxicity between saline diluted drug and the citrated solution. The citrate buffer was chosen as a solvent to avoid precipitation of the drug in plasma. The drug, formulated as a 100 mg vial, is undergoing Phase I clinical trial at the National Cancer Institute, Bethesda, Maryland.

5. Sodium Bromebrate (Cytembena, NSC 104801) This drug is a synthetic cytostatic agent originally developed in Czechoslovakia. It is commercially available in that country where it has undergone clinical studies in the treatment of gynecological malignancies and in surgical-adjuvant studies for various musculoskeletal sarcomas. Sodium bromebrate does not show activity in the L1210 experimental tumor system, but has been active in such experimental systems as sarcoma 180, the Erlich ascitic carcinoma, and the Yoshida ascitic sarcoma, among others. The drug is thought to exert its antitumor activity through inhibition of tetrahydrofolate formylase and, thus, indirectly purine ring biosynthesis. It is also thought that the drug prevents incorporation of amino acids into proliferating cells. Acute toxicity studies indicate an LD50 = 50 mg/kg (IV route) in the mouse, and an LD50 = 245 mg/kg by this route in the rat. Chronic toxicity studies in the latter species indicate no significant hematologic changes, nor alterations in liver and renal function tests. Also, no signs of toxicity have been found after administration of the drug to only five dogs. Pre-clinical toxicology studies with drug synthesized and formulated in the United States are currently conducted by the NCI. Phase I clinical trials have started at the Mayo Clinic, and at Roswell Park Memorial Institute. The drug is formulated as a 200 mg vial for clinical use.

6. Ftorafur (NSC 148958) This drug is a fluorinated pyrimidine antimetabolite synthesized in the Soviet Union in the mid-1960s. The antitumor activity of ftorafur, both experimentally and clinically, is similar to that of 5-FU. The overall minimal toxicity of ftorafur relative to 5-FU would appear to be a clinical advantage. From a pharmacologic point of view, it appears ftorafur acts as a depot form of 5-FU, thus providing a continous low-dose infusion of 5-FU. Ftorafur is probably a weak inhibitor of DNA thymine and RNA pyrimidine. In the Soviet Union, ftorafur has shown the following broad spectrum of experimental antitumor activity comparable to that of 5-FU.

Tumor System	Percent Inhibition	
	Ftorafur	5-FU
Sarcoma 180	60	59
Sarcoma AK	53	61
Melanoma (H-P)	58	55
Walker carcinoma	63	55

In toxicology studies with mice and rats, ftorafur had about 1/6 the toxicity of 5-FU when single doses of drug were administered orally, IV, IP, and SC. When single IV doses of ftorafur and 5-FU were given to mice, both showed similar effects upon platelets and RBCs, i.e., significantly decreased numbers of platelets after day 2 with return to normal counts of both platelets and RBCs by day 60. At present the NCI is conducting preclinical toxicology studies in dogs using drug synthesized and formulated in the United States. Phase I clinical trials are being carried out at M. D. Anderson Hospital and Tumor Institute.

7. BCG (NSC B116327, Tice, and NSC B116328, Pasteur) THE NCI is sponsoring clinical trials with vaccine obtained from two different sources: Tice (or Chicago strain) and Pasteur. Both are lyophilized preparations and differ in the number of viable organisms per ampule. Typically, the Tice strain contains $2\text{-}8 \times 10^{8}$ viable units, wheras the Pasteur strain is reported by the manufacturer to contain 3.75×10^{7} to 1.5×10^{8} Colony Forming Units. In addition, the Pasteur BCG may be still lower, by as much as two log units. Published data in the literature indicates that Tice BCG given by scarification on an intensive schedule decreases the postoperative relapse rate and prolongs the disease-free interval and survival for patients with malignant melanoma. At present, the Southwest Oncology Group is comparing chemotherapy with and without Pasteur BCG in adult leukemia.

8. S-Trityl-L-Cysteine (NSC 83265, 3-tritylthio-L-alanine) This drug is a synthetic analogue of cysteine, which is a non-essential amino acid for normal cells grown in culture. Reports in the literature indicate that cysteine is absolutely required to facilitate growth of leukemic leukocytes in cell culture and leukocytes of acute leukemia have a greater avidity for L-cysteine than normal leukocytes. The clinical dosage form of this drug shows moderate activity in the L1210 experimental tumor system. An optimal dose of 10 mg/kg administered IP on an intermittent schedule of Q3h, Q4d, d1, 5, 9 shows a %ILS of 51, whereas an optimal dose of 40 mg/kg by this route on a chronic schedule of 9 days shows a %ILS of 38. It has been suggested that the drug exerts its action by inhibition of RNA synthesis and protein synthesis. Also, a possible mechanism of action involves inhibition of glyoxalase activity in dividing cells. In preclinical toxicology studies in dogs and monkeys, the major organ toxicities included the liver, kidney, hematopoietic, and GI tract. Liver toxicity, as manifested by an elevation in serum bilirubin, was observed only in the monkey. This species was also more sensitive to renal damage than the dog. However, the dog was more susceptible to GI toxicity compared to the monkey. The drug is formulated for clinical use as a 50 mg vial, and is undergoing Phase I trial at the Children's Cancer Research Foundation in Boston.

The Drug Liaison and Distributuion Section is also preparing IND applications for 11 other anticancer agents, 7 of which constitute original applications. The originals include:

1. Anguidine (NSC 141537) This fermentation antibiotic drug shows activity in the L1210 and P388 experimental tumor systems.

2. Indicine-N-Oxide (NSC 132319) This drug has activity in P388 leukemia and in B16 melanoma. No activity has been observed against L1210 or the Lewis lung.

3. 2-Amino-1, 3, 4-thiadiazole (NSC 4728) This synthetic antimetabolite shows activity in the L1210 system with a %ILS > 150. The drug was put into clinical trial in the late 1950s with at least 13 patients being treated. All patients experienced a marked rise in serum uric acid and urinary acid excretion, and no antitumor activity was noted. Because of the experimental antitumor activity and the belief that this drug did not receive an adequate clinical trial, an IND application will be prepared to reopen clinical trials.

4. Galacticol (NSC 132313) This drug, which has undergone extensive preclinical development in Hungary, shows a marked inhibition of tumor growth in L1210 leukemia, sarcoma 180, and Erlich ascites tumor. It has been suggested that this drug is a metabolite of dibromodulcitol (NSC 104800) and its structure indicates that it may act as an alkylating agent.

5. Neocarzinostatin (NSC 157365) This drug is a polypeptide which has undergone preclinical development and clinical trials in Japan. An IND application based upon the Japanese clinical studies will be submitted in order to initiate clinical trials in the United States.

6. Fluorodopan (NSC 73754) This drug is the second of a group of 3 drugs which the Soviet Union sent to the United States. Structurally, it is an analogue of uracil mustard and is thought to act as an alkylating agent. The drug shows activity in the B16, P388, and W256 experimental tumor systems, but it is not active against the Lewis lung and sarcoma 180. In animal toxicology studies conducted in the Soviet Union, only small animal species (mice, rats, and rabbits) were used. A range of toxicities were reported including cardiovascular, CNS, renal, and hematologic. Prelinical toxicology trials in the dog are currently being undertaken by the NCI in preparation for an IND appliation.

7. C. parvum (Corynebasterium Parvum, NSC 197213) C. parvum is the third immunoadjuvant agent for which an IND application will be prepared. In experimental tumor systems, C. parvum has demonstrated enhancement of resistance to neoplasia in AKR leukemia, L1210, Erlich ascites in Swiss mice, and Moloney virus leukemia. Clinical trials in the U. S. will employ two sources of the drug, i. e., the so-called Merieux strain from France and the one produced by Burroughs-Welcome.

IND applications will also have to be made in the form of amendments to pre-existing INDs for the following 4 drugs:

1. Procarbazine (NSC 77213) This drug is indicated for oral administration, and clinical trials by the intravenous route will be started.

2. Methyl CCNU (NSC 95441) At present, clinical trials with this drug of the nitrosourea family will be expanded to include parenteral studies with another vehicle (Emulfor).

3. Calcium Leucovorin (Citrovorum Factor, NSC 3590) It is anticipated that clinical trials under IND sponsored by NCI will include a solid dosage form for the oral route of administration.

4

4. 5-FU (NSC 19893) A new, solid dosage form of this drug has been developed by a pharmaceutical firm, and it is anticipated that clincal trials by the oral route will be started.

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